Infrared microspectroscopy

Christophe Sandt
SMIS beamline
Synchrotron SOLEIL

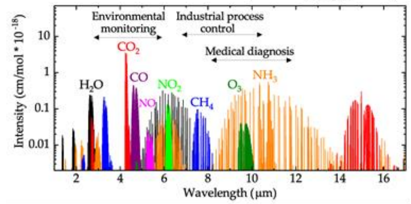
Plan

- Introduction
  - FTIR spectroscopy
  - FTIR microspectroscopy
- Techniques
- Applications
- Beyond the diffraction limit
- Data Analysis

IR in SCIENCES

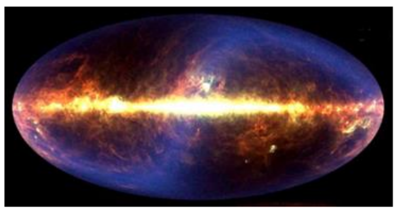
From parsec to nm

Atmospheric analysis
km²

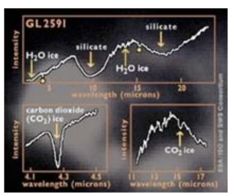


Agilent 4300
Handheld spectrometer

Few mm²

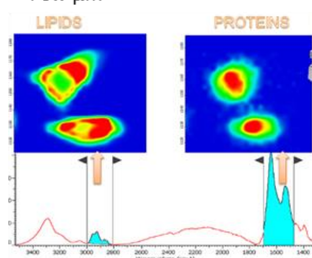


The Infrared Sky
January 28, 1998
DIRBE Team, COBE, NASA

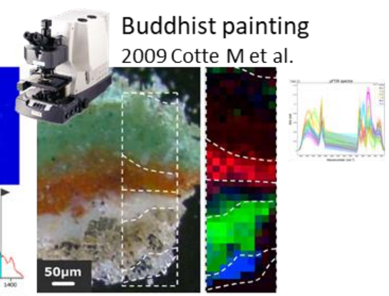


Warm dust ~100 µm
Cool stars energy peak ~ 1µm
Giant planets ~ 6-15µm
PAH 6 µm, silicates 10 µm
Dust re-radiation ~ 20-200µm

Single HeLa cell
2008 Chio-Srichan S et al.
Few µm²

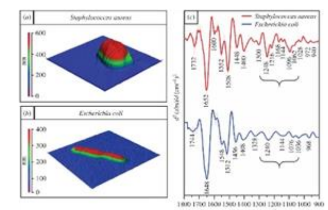


Buddhist painting
2009 Cotte M et al.

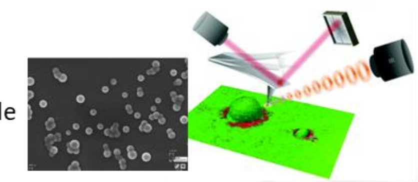


Single bacteria
2018 Kochan et al.
Interface

1000 m



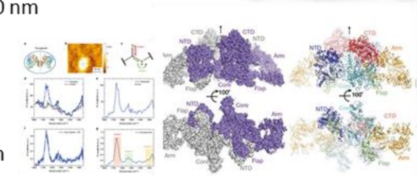
Mid IR spectroscopy is used across multiple scales & sciences



Single nanoparticle
2018 Mathurin et al.
Analyst

10-20 nm

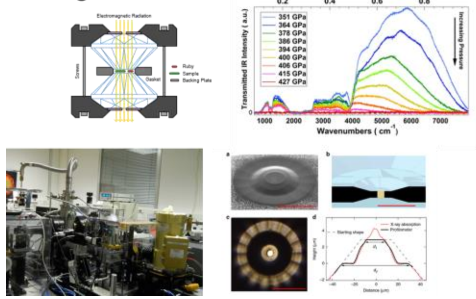
Single protein
2020 Ruggieri et al.
Nature Comm, 11
Few nm



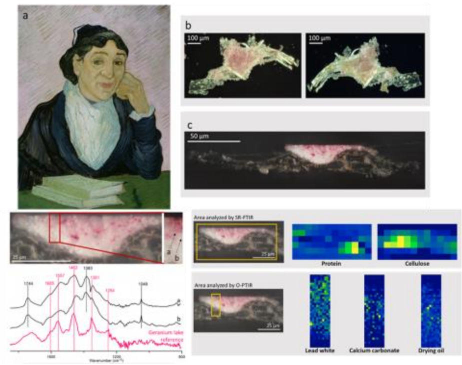
INFRARED MICROSPECTROSCOPY @ SYNCHROTRONS

Materials in extreme conditions

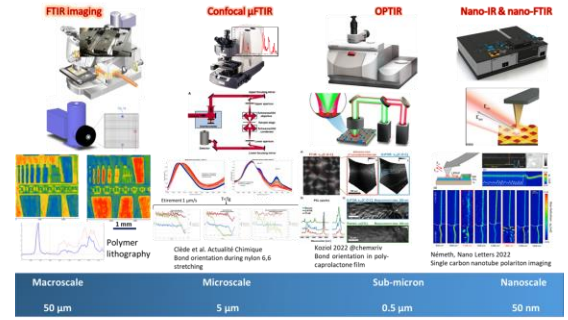
High Pressure



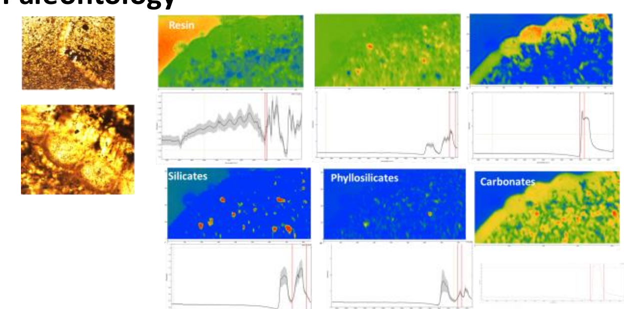
Cultural heritage



Polymer sciences

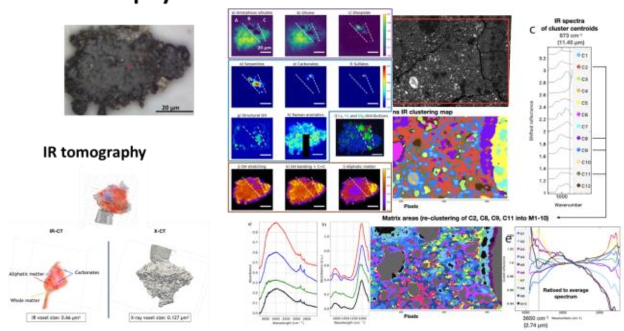


Paleontology

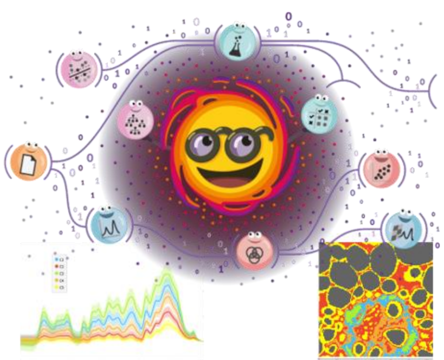


Astrophysics

IR tomography

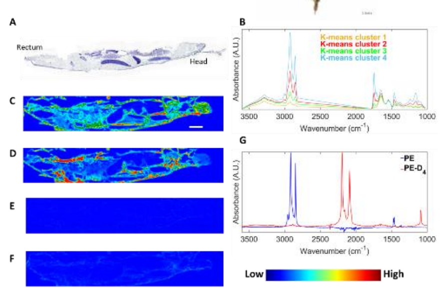


Data analysis

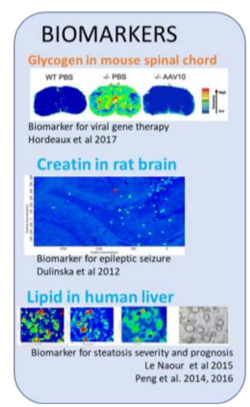


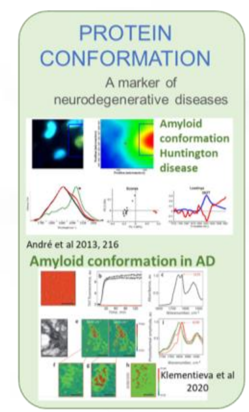
Quasar software
https://quasar.codes

Environnement



Biology and Biomedical



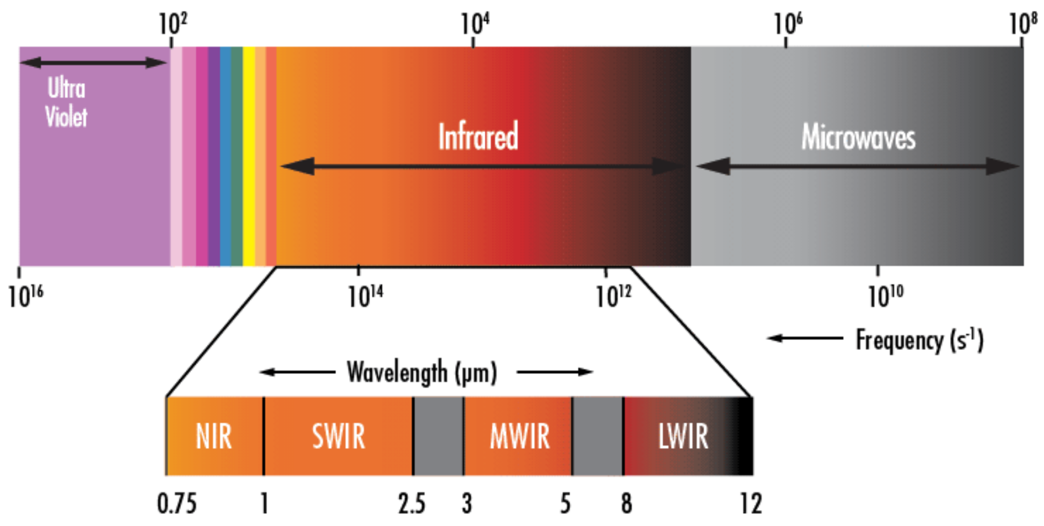


The IR domain

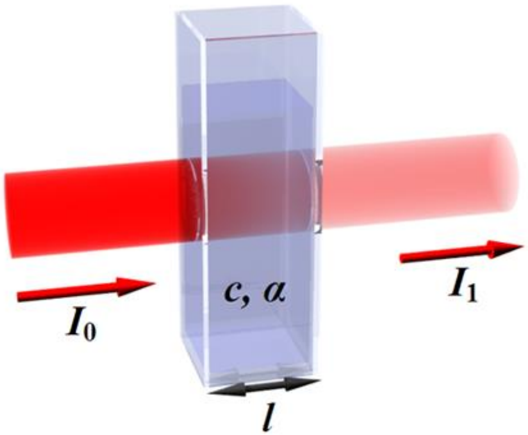
- Optical method in the IR range
  - "Light*"-matter interaction
  - Near, mid, far IR:

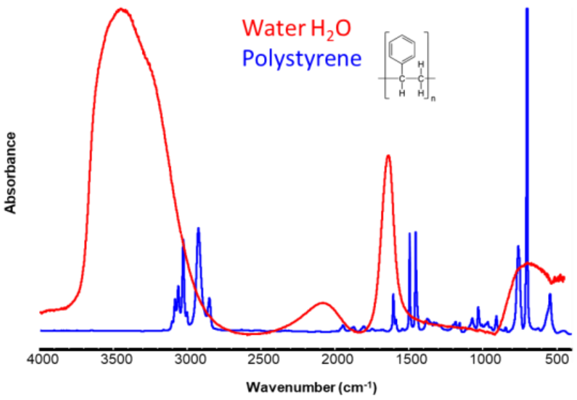
* actually IR radiation, not light

Domain	Wavelengths	Wavenumbers
Near IR	0.8-2.5 µm	1250 – 4000 cm^{-1}
Mid IR:	2.5-25 µm	4000 – 400 cm^{-1}
Far IR	25-1000 µm	400-10 cm^{-1} .



- Absorption spectroscopy





Beer Lambert Bouguer law

$$A = \varepsilon c l$$

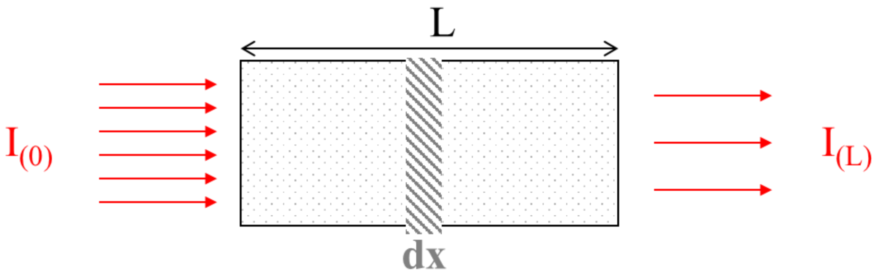
A= Absorbance

ε= molar absorptivity ($cm^{-1} \cdot L \cdot mol^{-1}$)

C= concentration ($mol.L^{-1}$)

l= pathlength (cm)

Derivation of the BBL law



$$-dI_{(x)} = KI_{(x)}cdx$$

$$-\left[\ln I_{(x)}\right]_{I_{(o)}}^{I_{(L)}} = \left[Kcx\right]_0^L$$

$$\ln\left(\frac{I_{(0)}}{I_{(L)}}\right) = KcL$$

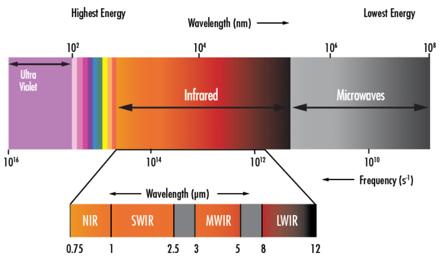
$$\frac{I_{(L)}}{I_{(0)}} = e^{-KcL}$$

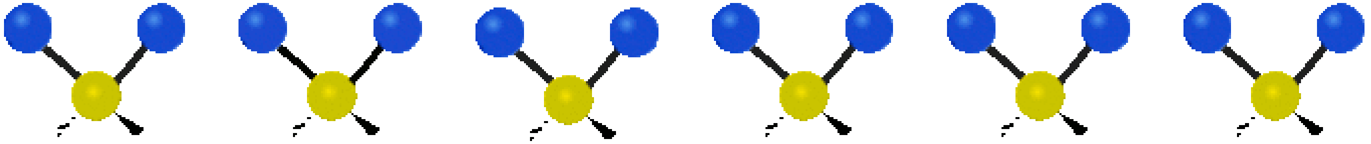
$$A = \log\left(\frac{I_{(0)}}{I_{(L)}}\right)$$

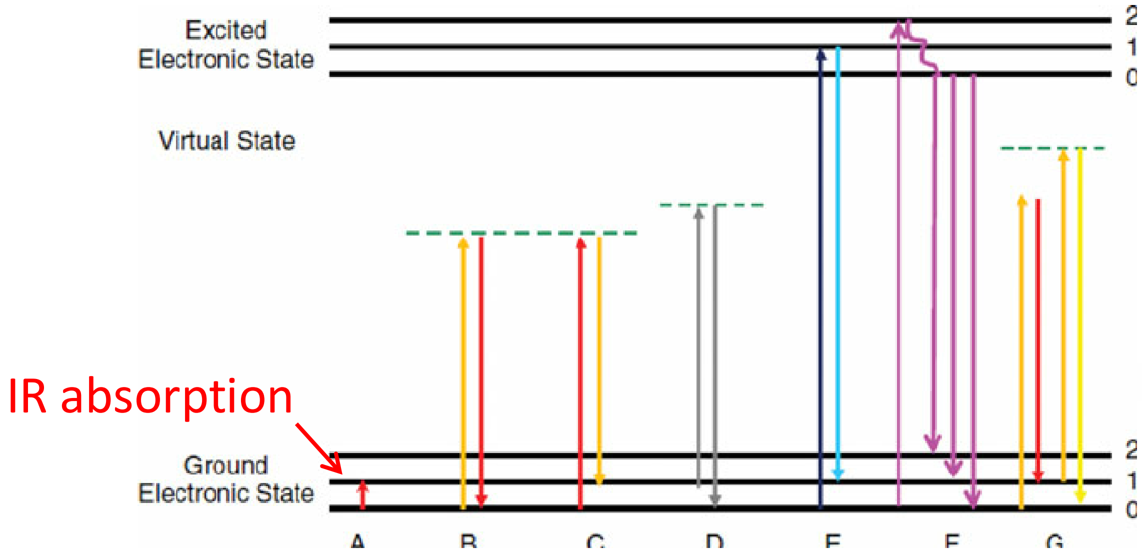
c: concentration
x: distance
I_0: incident beam Intensity
I_L: transmitted beam Intensity

FTIR spectroscopy

- Vibrational spectroscopy
- Probes vibrations of molecular bonds
  - Actually **Ro**vibrational: probes vibrations **and** rotations





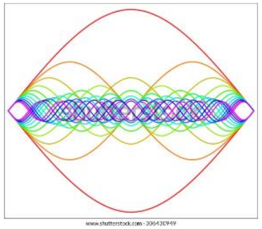


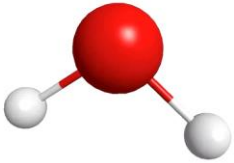
FTIR spectroscopy

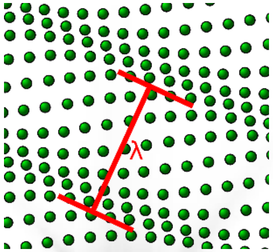
- Bond vibrations
- Different energy ranges probe different vibration types:
  - Near IR :0.8-2.5 µm or 12500 – 4000 cm^{-1}: overtones of fundamental vibrations . Low absorptivity, low sensitivity long penetration pathlength.

  - Mid IR: 2.5-25 µm or 4000 – 400 cm^{-1}: fundamental vibrations and combinations. High molar absorptivities, high sensitivity, low penetration.

  - Far IR: 25-1000 µm or 400-10 cm^{-1} : low-energy modes, skeletal vibrations, phonons.

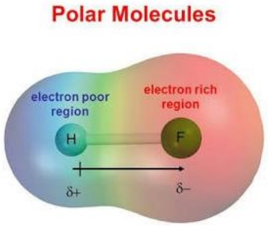




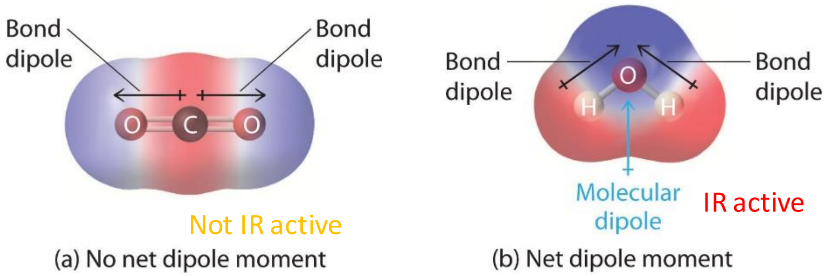


FTIR spectroscopy

- IR Active vibrations: permanent dipolar moment



- IR absorption is proportional to the stength of the dipolar moment

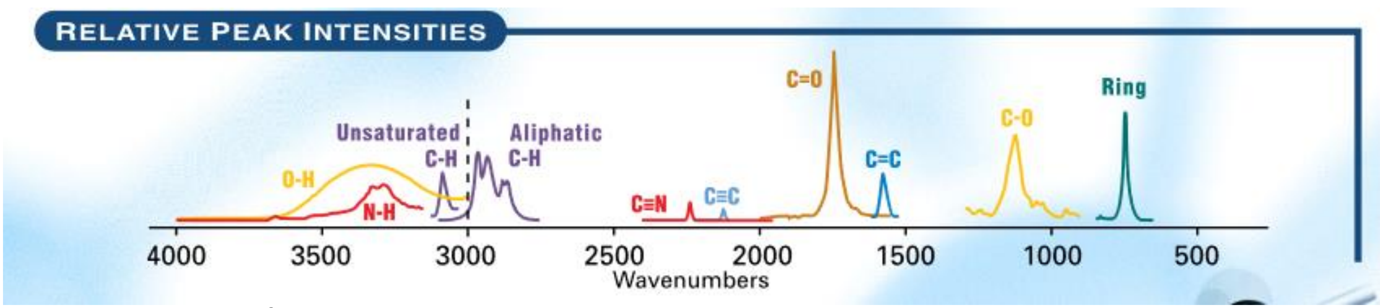


- Symmetric bonds/molecules are not active
  - IR spectroscopy is linked to molecular symmetry
  - C-C and C=C are almost inactive

FTIR spectroscopy

- Peak position depends on bond strength
  - Atom masses and bond conformation
  - Single/double/triple bond
  - Isotopic labelling

$$\sigma = \frac{1}{2\pi c}\sqrt{\frac{k}{\mu}}$$



Large mass differences
O-H N-H C-H

Triple bonds

Double bonds

Single bonds

www.thermoscientific.com/ftir

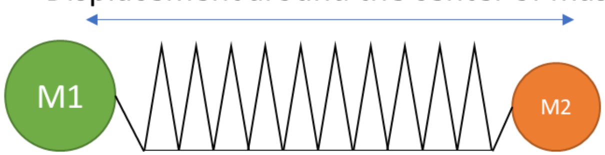
XX51346_E 02/15M

- Peak width depends on the organisation/phase:
  - Gaz: narrow peaks
  - Conformational entropy
    - Crystalline samples: narrow peaks
    - Amorphous samples : large peaks
  - Hydrogen- bonding increases peak width

Derivation of the peak position in the harmonic aproximation

Vibration frequency f

Displacement around the center of mass: x



Force constant k

There is two ways of calculating f:

- Solve the derivative of E_0 versus time which is equal to zero since E_0 is conserved
- Or solve ma=-kx

$$\vec{F} = -kx$$
$$\vec{F} = m\vec{a}$$

Total Energy: $E_0 = E_c + E_P$

E_c=1/2mv²

E_p=kx

E_0=1/2mv²+kx

E_0 is conserved: $\frac{\partial E_0}{\partial t} = 0$

a: acceleration

E_0: total energy

E_c: kinetic energy

E_P: potential energy

$$m\vec{a} = -kx$$
$$m\frac{\partial^2 x}{\partial t^2} + kx = 0$$

Solution of the differential equation:

x(t)=Acos(ωt)

with ω the angular frequency: ω=2πf with f the oscillation frequency

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

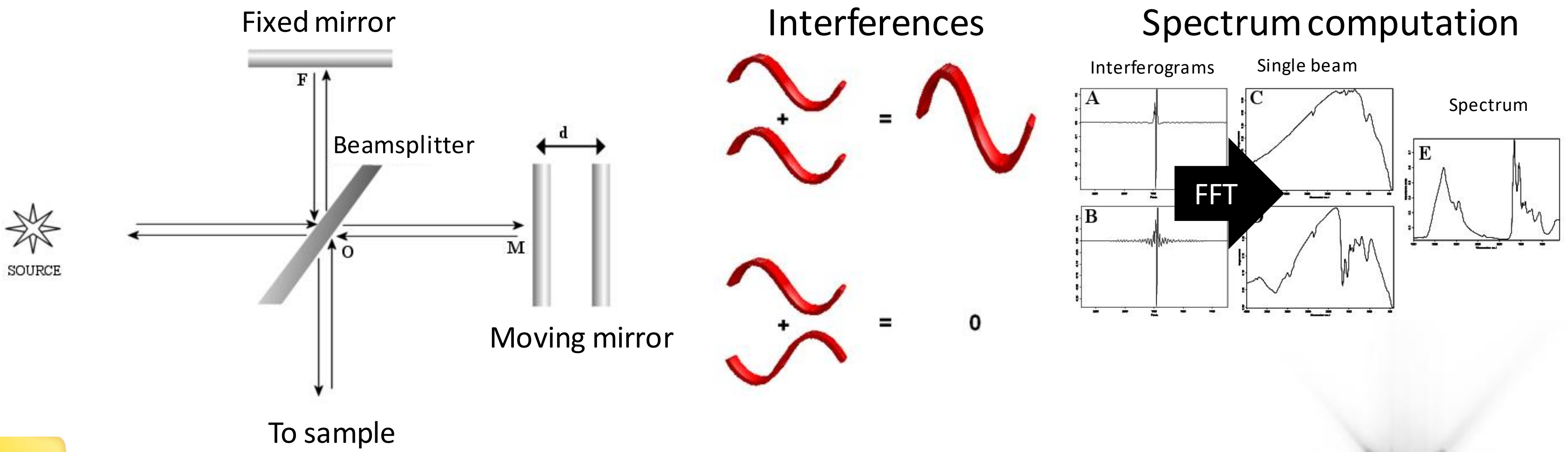
K: force constant

µ: reduced mass

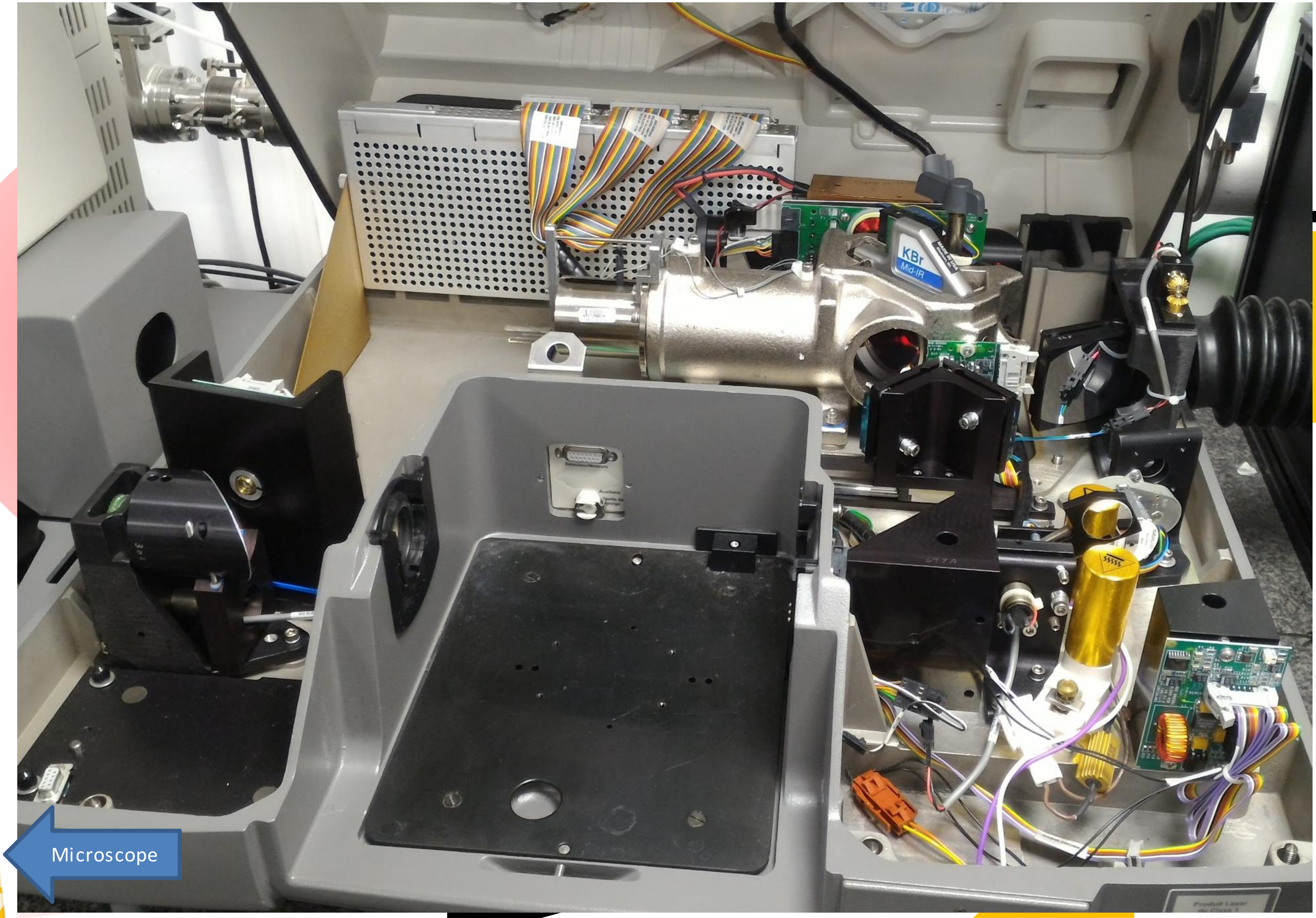
$$\mu = \frac{M_1 M_2}{M_1 + M_2}$$

FTIR: Interferometry

- An interferometer is used to separate wavelengths by creating interferences in the IR beam
- Fourier Transform (FT) is the mathematical operation used to retrieve the separate wavelengths

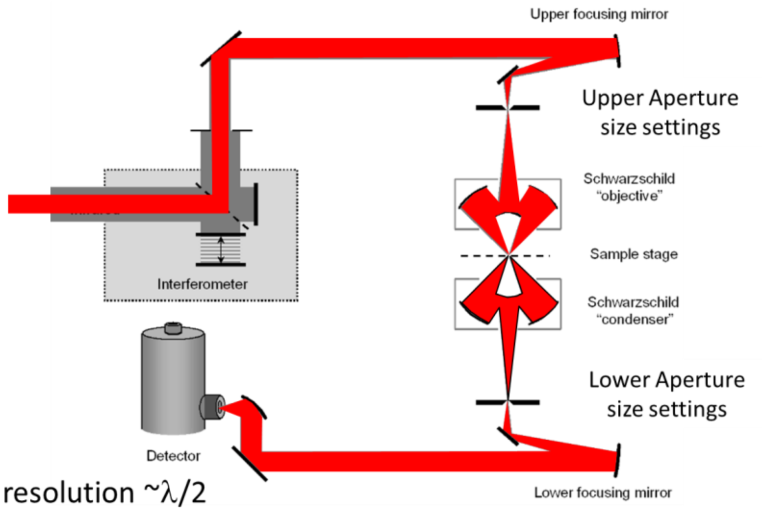


FTIR spectroscopy



FTIR microspectroscopy

FTIR microspectroscopy



Lateral resolution ~λ/2

The IR microscope

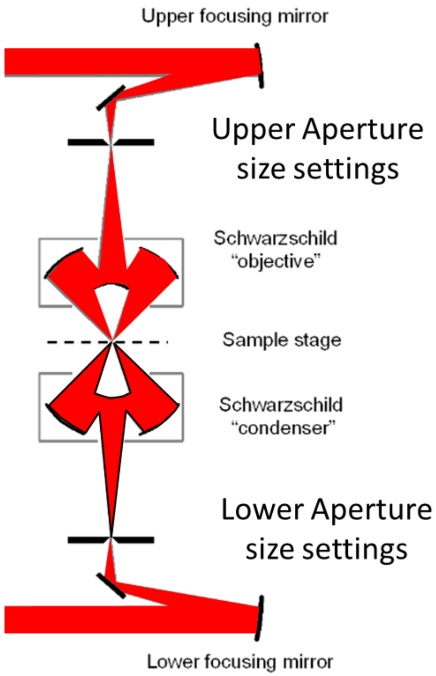
- Used to measure small samples
- Focus the IR beam to a small spot
- Use Semi-confocal or Confocal apertures to select the measurement area

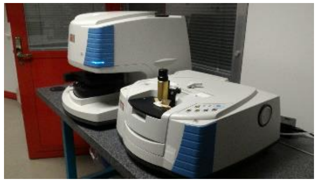
- All reflective objectives
  - 15X, 25X, 32X, 36X… enough to reach diffraction limit
  - Cassegrain
  - Schwarzschild

- IR detectors:
  - MCT narrow band 650-10,000 cm-1 LN_2 cooling
  - MCT wide band 400-10000 cm-1 LN_2 cooling
  - InSb (2000-10,000 cm^{-1})
  - Bolometer (50-1000 cm^{-1}) **He cooling**

- Motorized stage
  - Computer controlled

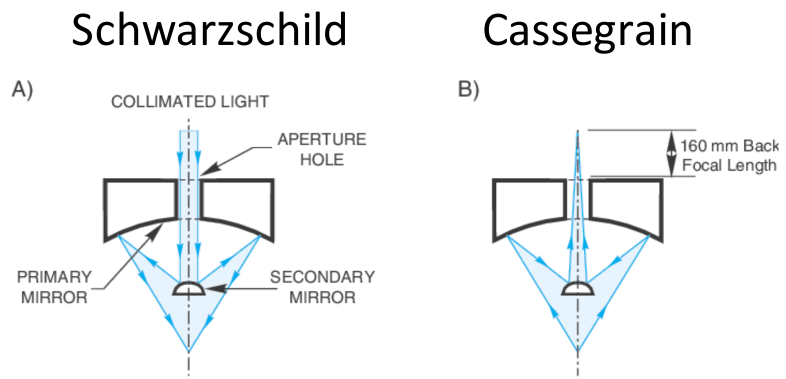
- Accessories
  - IR and/or visible polarization
  - Fluorescence imaging

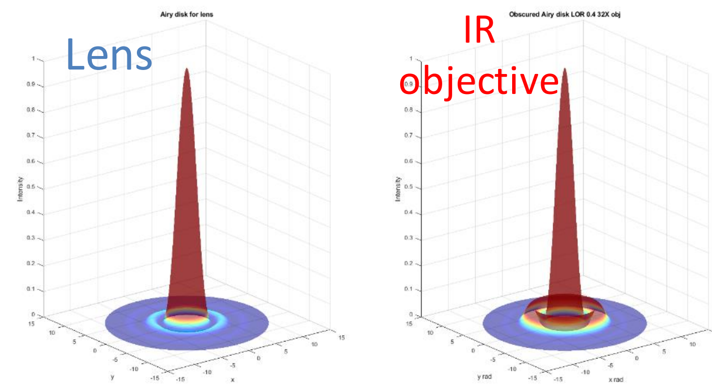




FTIR microspectroscopy

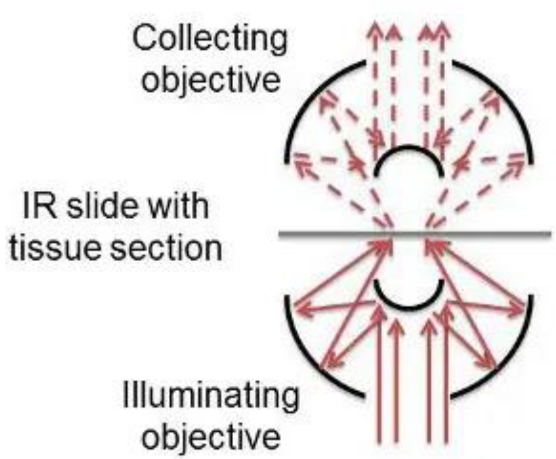
- IR objectives
  - All reflective spherical objectives
    - Avoid chromatic aberrations over the whole spectral range
  - Schwarzschild: focus to infinity
  - Cassegrain: focus to a focal point
- Central obscuration
  - Reduced throughput
  - Reduced resolution
  - Lower energy in the central lobe
  - Higher energy in the lateral lobes
- Objective magnifications
  - 15X
  - 25X, 30X, 32X, 36X, 40X
  - N.A. 0.5 - 0.8



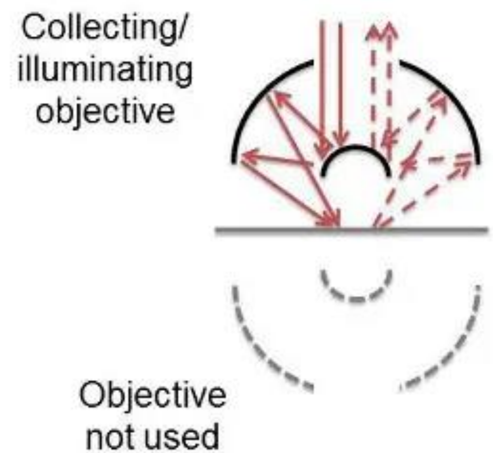


- Measurement modes

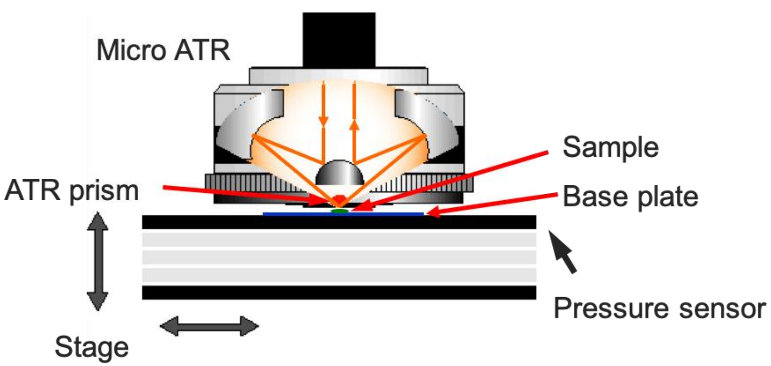
A) Transmission-mode



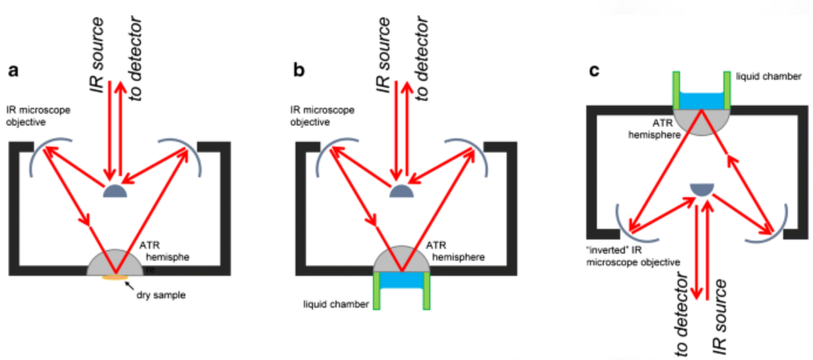
B) Reflection-mode



C) micro ATR mode

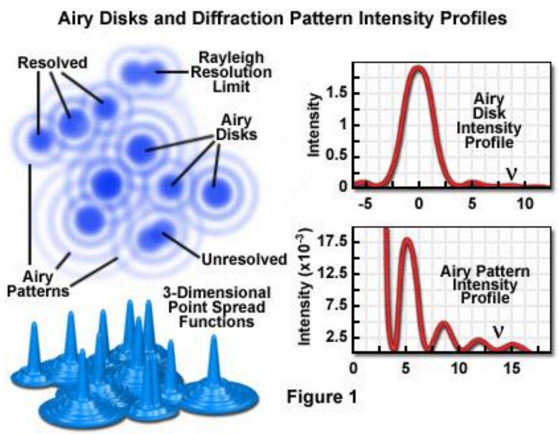


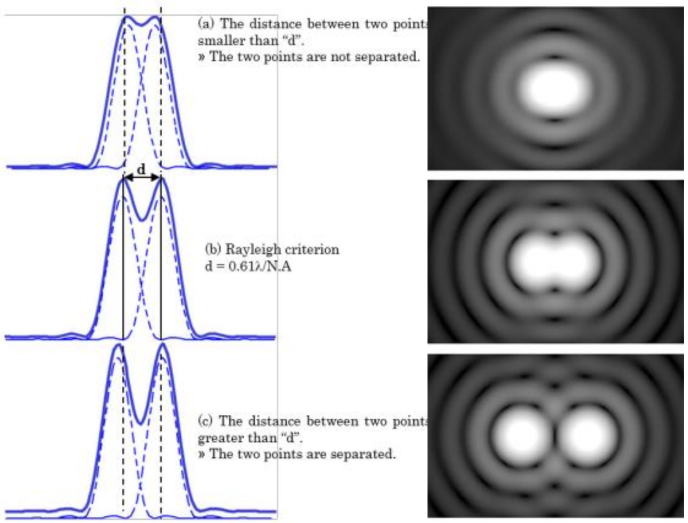
D) Inversed micro ATR mode



FTIR microspectroscopy

- Spatial resolution





- Limited by:
  - Wavelength and diffraction limit: 2.5-25 µm in the mid-IR
  - Signal to noise ratio: source brilliance (nb of photon/unit angle)
  - Optical system (confocal arrangement; objective type, numerical aperture and obscuration ratio, alignment quality, measurement mode…)
  - Sample (refractive index, thickness, geometry, chemical contrast…)

FTIR microspectroscopy

- Theoretical diffraction limit: given by Abbe equation:

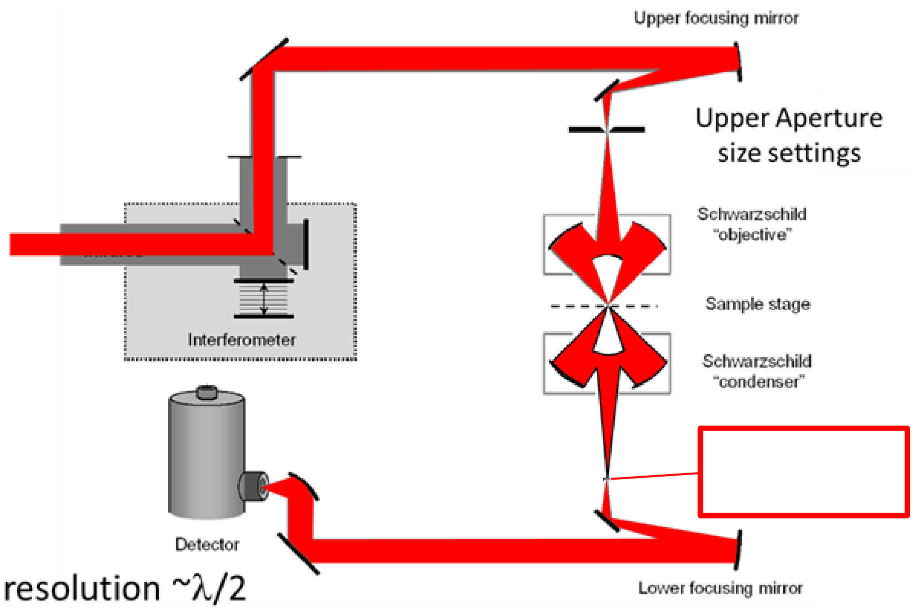
$$R = \frac{0.61\lambda}{NA}$$

R: resolving power
λ: wavelength (μm)
NA: objective Numerical Aperture, NA= nsin(θ)

- Actual resolution will be worst due to:
  - Diffraction from sample/optics
  - Objective obscuration ratio
  - Alignment
  - In reflection mode

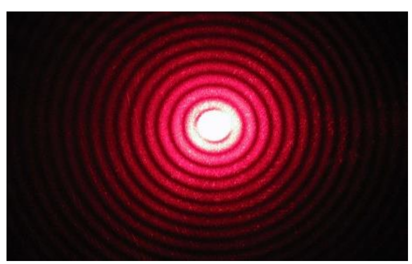
Typical NA for IR objectives:
15X objective: ~0.5
30X objective : ~0.6

FTIR microspectroscopy

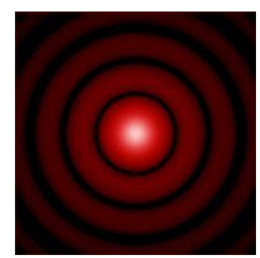


Lateral resolution ~λ/2

Spatial resolution limit: diffraction



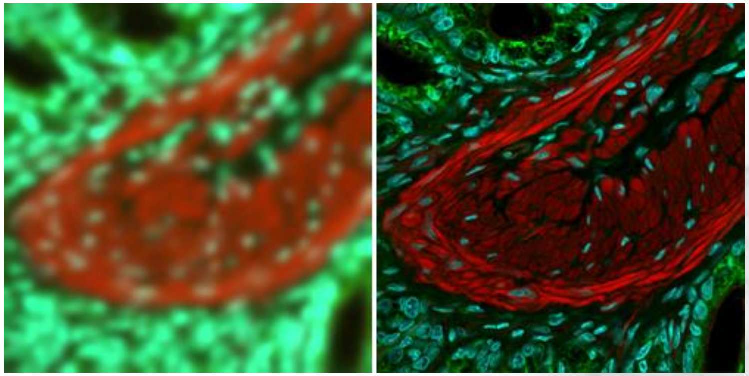
Diffraction pattern by a single pinhole



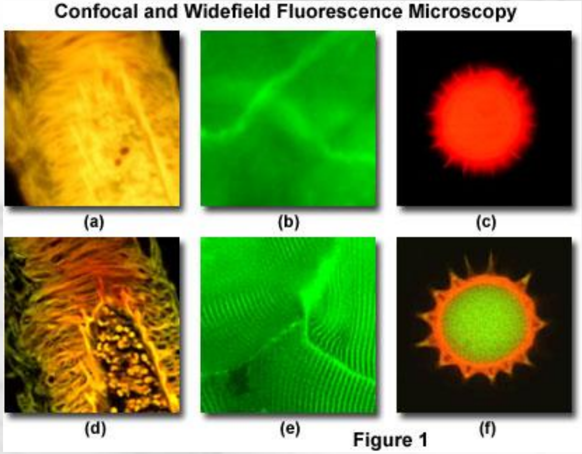
Diffraction pattern in a confocal system

Lateral resolution: ~λ/2

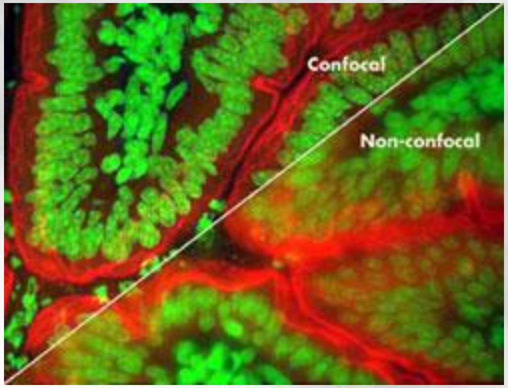
CONFOCALITY

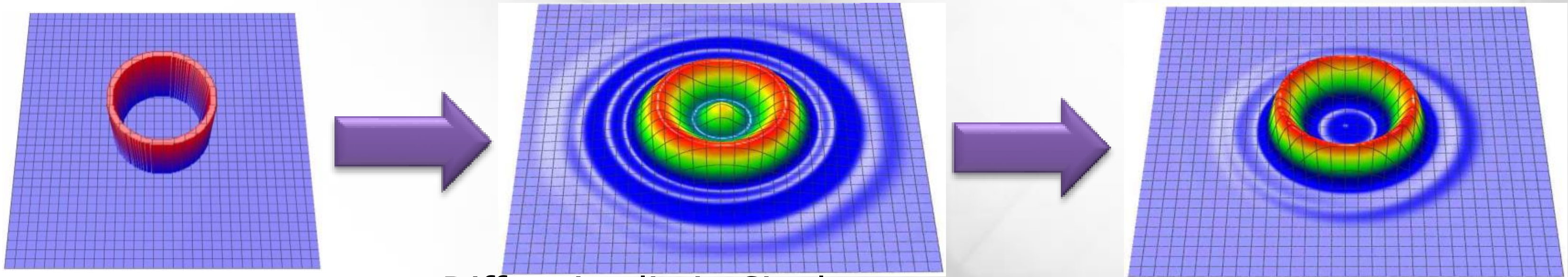


Olympus - Life Science Solutions



Atto Bioscience

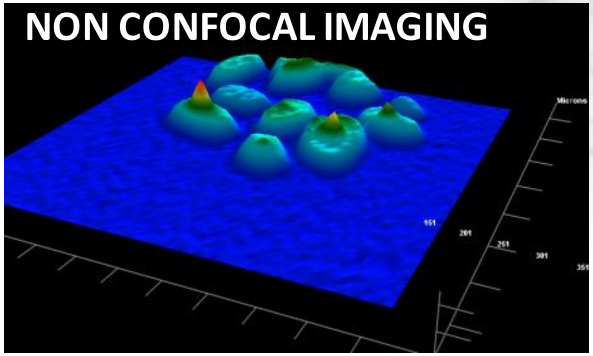


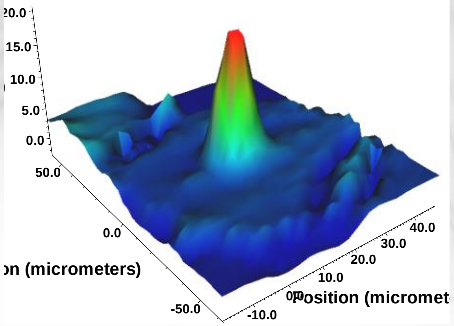


Simulation by Paul Dumas

Diffraction limit: Single aperture

Diffraction limit: Confocal apertures

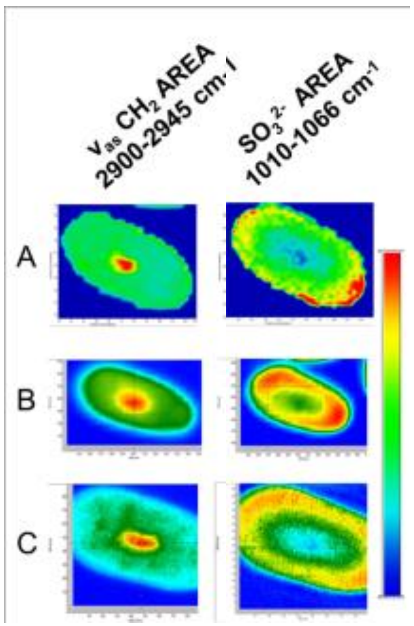


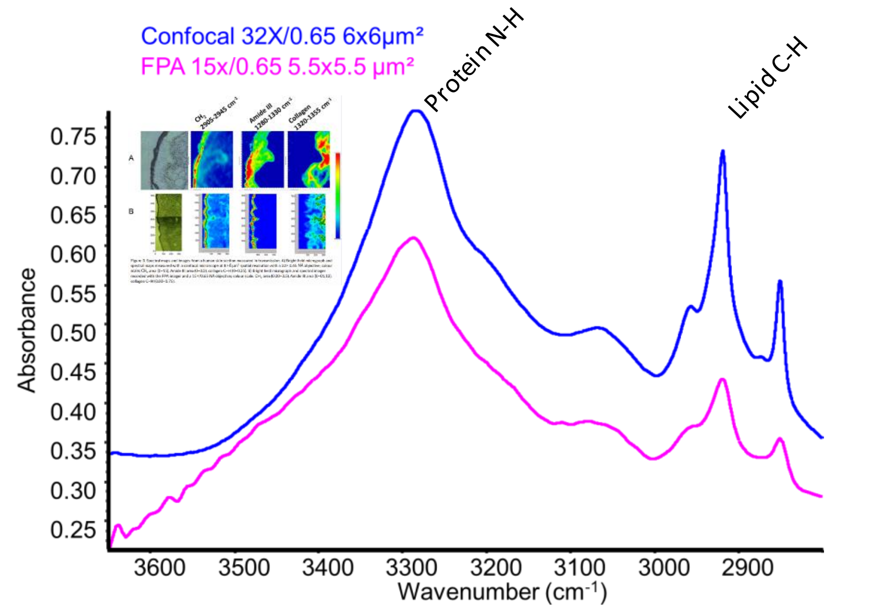


SYNCHROTRON WITH CONFOCAL MICROSCOPE

Importance of spatial resolution:
accuracy of the chemical information

C-H/N-H peak ratio from the same particle are inversed between a highly resolved confocal instrument and an non-confocal imaging system





Spectral images and maps of the same hair medulla

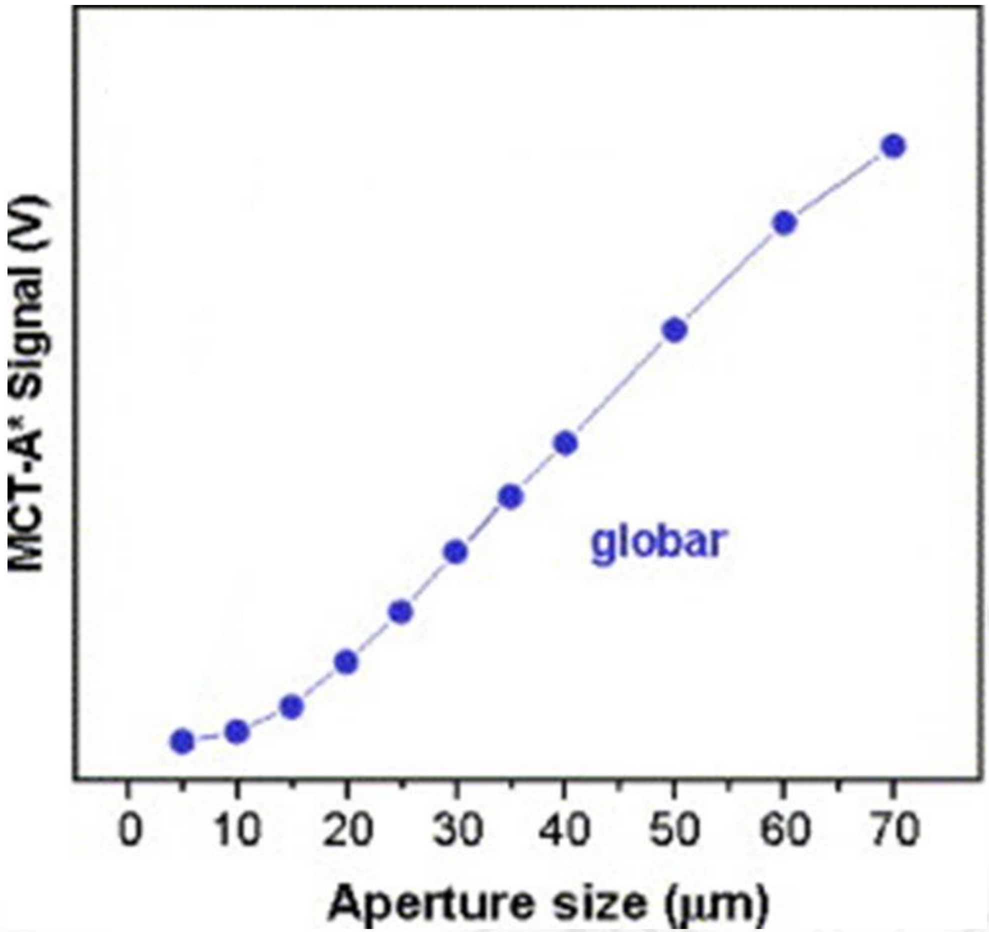
A. Confocal microscope with synchrotron source
B. Low magnification imaging system (15x)
C. High magnification imaging system (62x)

Spectra of the medulla of the same skin stratum corneum layer measured with either:

- A confocal microscope with synchrotron source at 6x6 µm² resolution
- A non-confocal IR microscope with FPA detector at 5x5 µm² projected pixel size

Sandt et al. Journal Spectral Imaging 2020

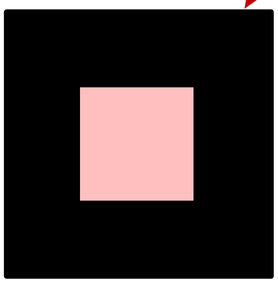
Signal versus confocal aperture size

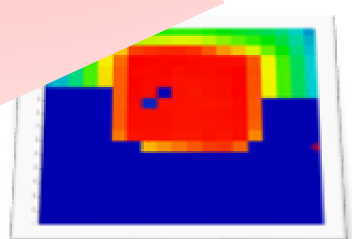


FTIR microspectroscopy

- Synchrotron source advantages

Thermal SOURCE





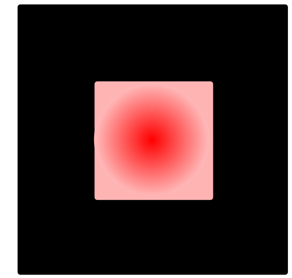
SPATIAL RESOLUTION
25-50 µm
LIMITED BY THE SOURCE BRILLIANCE

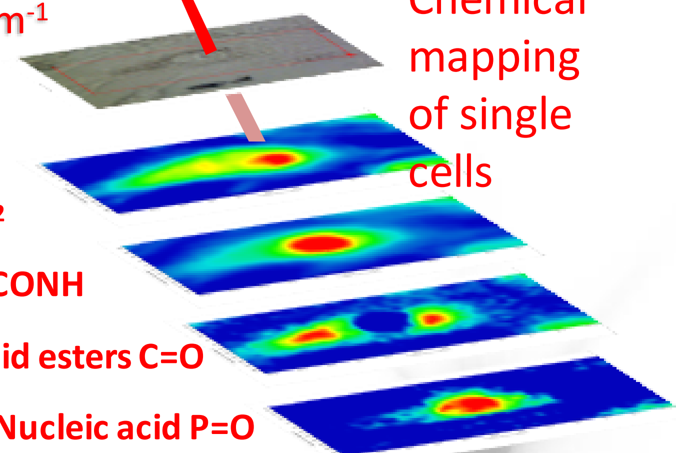
SPECTRAL RANGE
400-7500 cm^{-1}

SYNCHROTRON SOURCE

SPATIAL RESOLUTION
DIFFRACTION LIMITED
3-15 µm RESOLUTION
CONFOCAL

SPECTRAL RANGE: BROADBAND
50-10000 cm^{-1}





Lipid CH_2

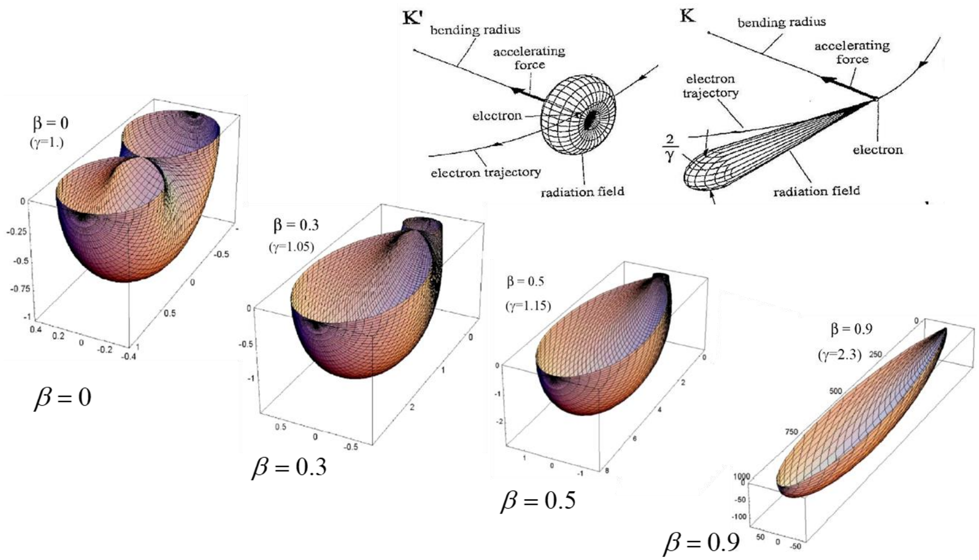
Protein CONH

Lipid esters C=O

Nucleic acid P=O

Chemical mapping of single cells

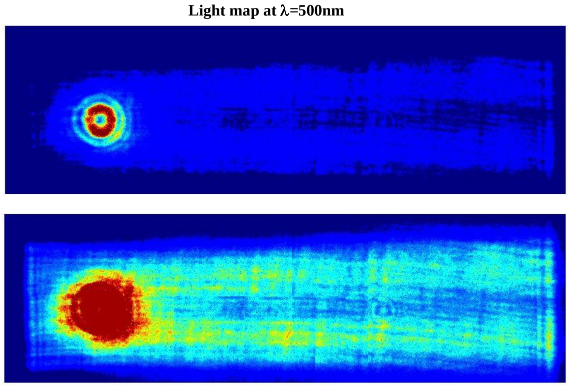
Synchrotron brilliance/brightness

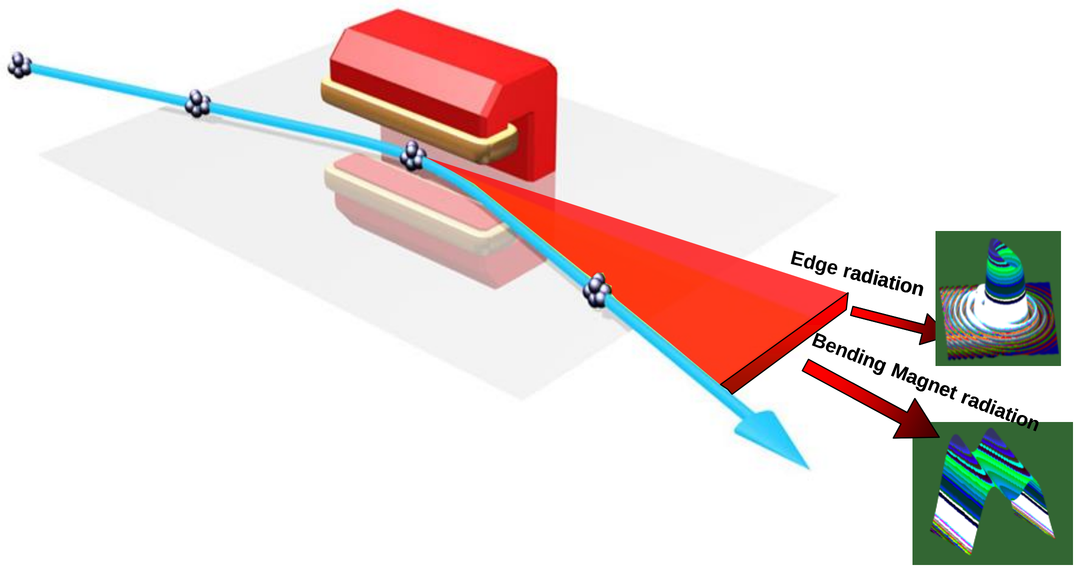


IR is generally collected with a slotted mirror from a **bending magnet** source:

2 types of radiation:
-Edge radiation

- Bending radiation





Polarization
Edge radiation: circular
Bending radiation: straight

SOLEIL SYNCHROTRON

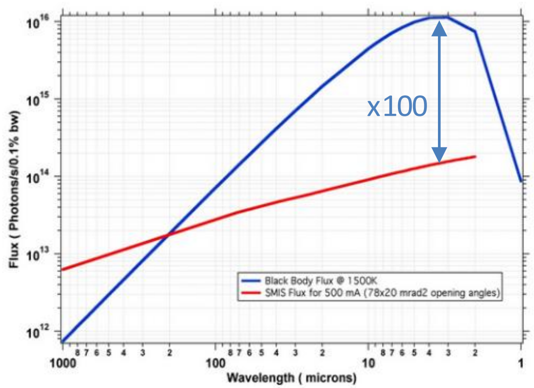
Synchrotron FTIR microspectroscopy

- Why use synchrotron source ?
  - Brilliance :

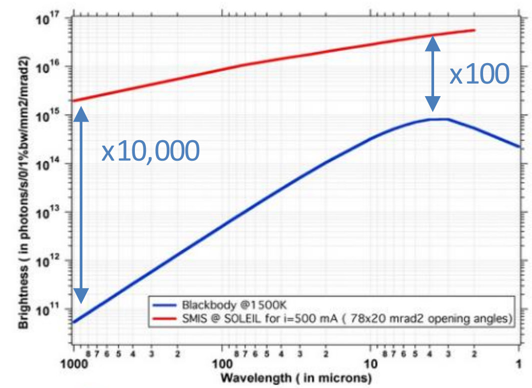
Black body thermal source (globar)

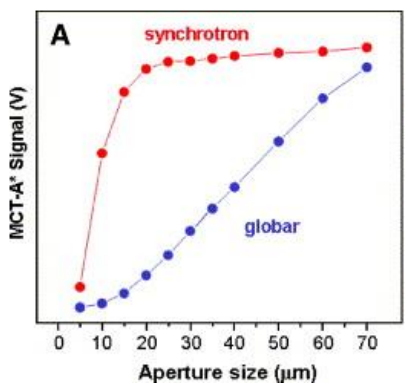
SOLEIL Synchrotron

Flux



Brilliance





Effect of the microscope aperture size on IR signal in the mid-IR

  - Spectral range: far-IR/ THz to near-IR

FTIR microspectrocopy

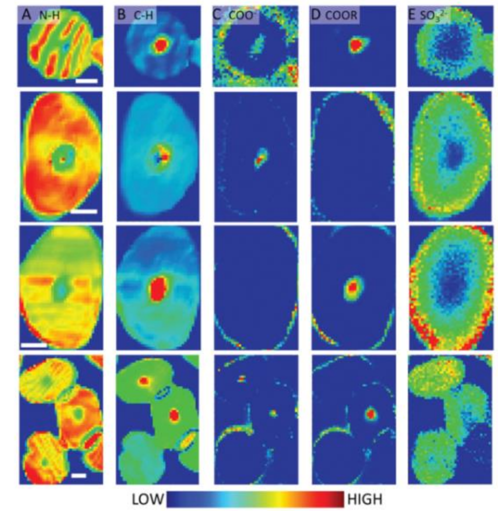
- Why use synchrotron source ?
  - To get relevant information on sample composition

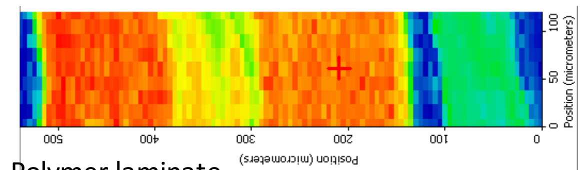
- QUALITATIVE ANALYSIS
  - NATURE OF MOLECULAR BONDS
  - FINGEPRINTING: SPECTRAL IDENTIFICATION IN DATABASES
- STRUCTURAL ANALYSIS
  - SENSITIVE TO BOND CONFORMATION
  - SENSITIVE TO BOND ENVIRONMENT (STERIC HINDRANCE, CONJUGATION, HYDROGEN BONDING…)
  - SENSITIVE TO BOND ORIENTATION
  - SENSITIVE TO ORDER (CRYSTALLINE PHASE, CRYSTALLINITY…)
- QUANTITATIVE ANALYSIS
  - BEER LAMBERT BOUGUER LAW: $A=\varepsilon c l$
  - RELATIVE CONCENTRATIONS: SEMI-QUANTITATIVE ANALYSIS
  - REGRESSION MODELS FOR COMPLEX MIXTURES
- ELECTRON ENERGY LEVELS
  - DENSITY OF STATES
  - FREE ELECTRON CARRIERS
  - BAND STRUCTURE
- SAMPLE EVOLUTION
  - NON DESTRUCTIVE
  - REACTION KINETICS
  - EFFECT OF TEMPERATURE, STRETCHING, PRESSION, IRRADIATION…
  - MATERIAL AGING
  - COUPLING WITH OTHER TECHNIQUES

Spatial information

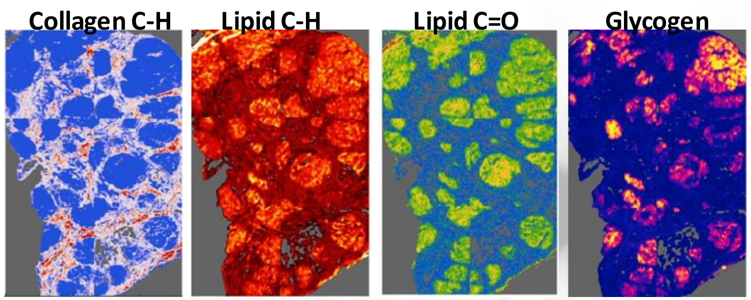
- ✓ Analysis of microparticles (1-100 µm) and fibers
- ✓ Differentiating mixtures, particles in matrices, multilayered samples
- ✓ Combination of the chemical and spatial information: chemical maps and images

Chemical maps of hair fibers with medulla





Polymer laminate



Liver cirrhosis tissue






µFTIR @ synchrotron beamlines

ALBA	MIRAS
ALS	BL1.4, BL2.4
Australian Sync	IR microspectroscopy
BESSY	IRIS
DIAMOND	MIRIAM
DAΦNE	SINBAD IR
ELETTRA	SISSI
ESRF	ID21
MAX IV	??
NSLS II	FIS
SESAME	EMIRA
SLRI	BL4.1IR
SOLARIS	CIRI
SOLEIL	SMIS
SPRING-8	BL43-IR







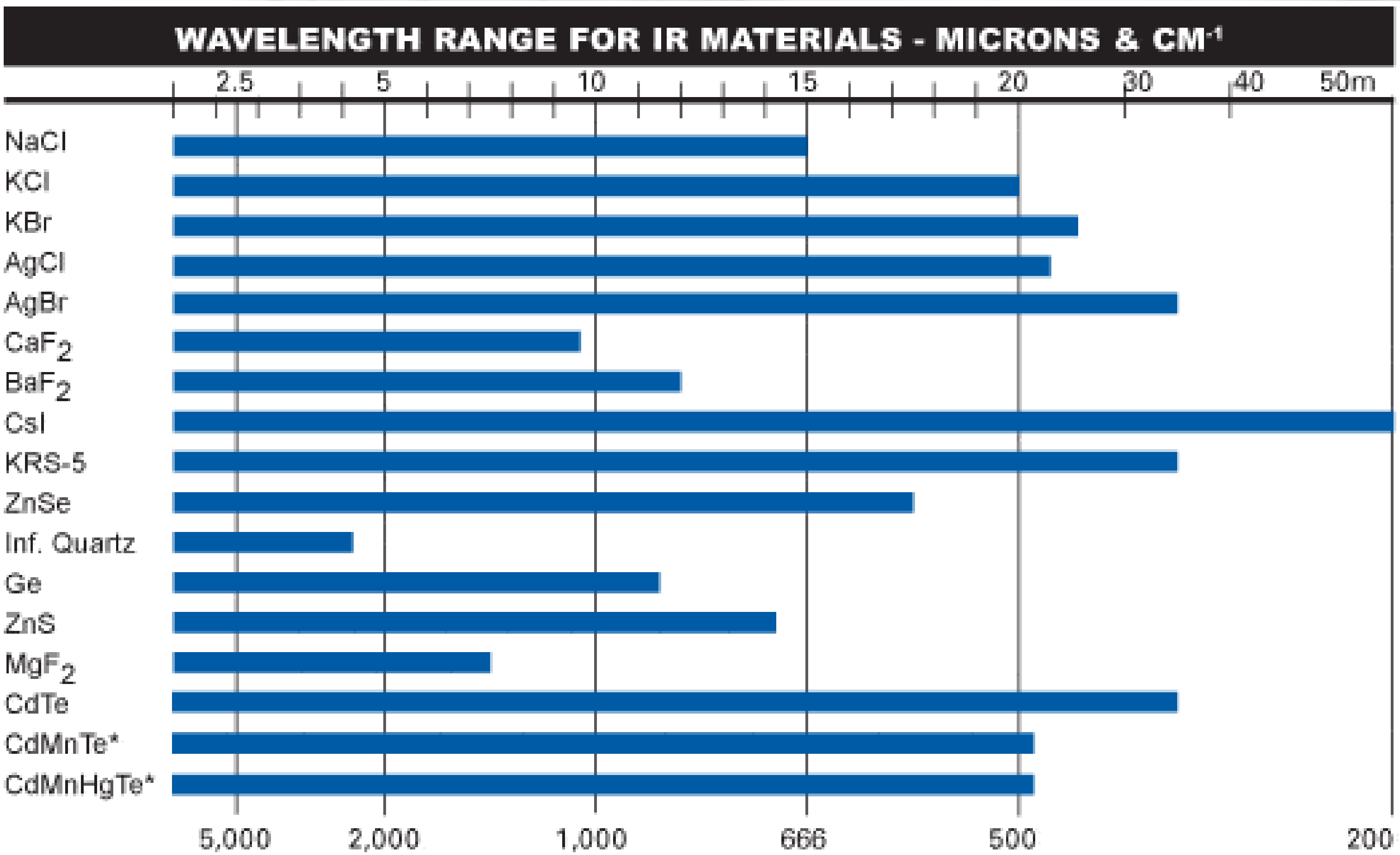


Samples and sample environments

- Thin samples:
  - Thin Films, Thin layers
  - Microtomed sections (1-25 µm)
  - Monocrystals
  - Particles, fibers
  - Thin powders: !! scattering, flattened powder grains
  - Cell cultures

- Sample environment
  - On transparent windows (BaF_2, C, CaF_2, Si, Si_3N_4, ZnS, ZnSe…)
    - Different properties, transmission ranges, surface properties, refractive indices, toxicity…
  - Reflective surfaces: gold, copper, low-e glass, ITO…
  - Transmission cells (temperature, atmospheric control, chemical reaction)
  - Microfluidic devices (for liquid samples and hydrated living cells)
  - Diamond compression cell (flattening samples)
  - Diamond Anvil Cells (DAC): high pressure

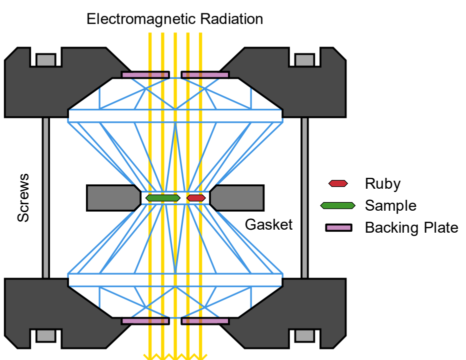
IR transparent materials

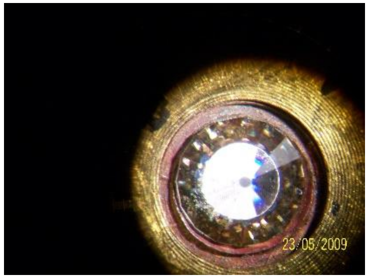


Samples and sample environments

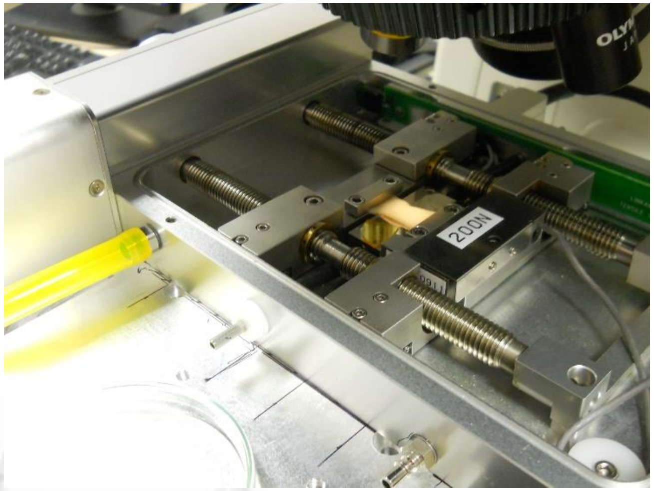
Diamond Anvil Cells

for measurements at high pressures





Stretching stage with temp control (350°C)



Microfluidic device

with temperature control (37°C)

Living biological cells

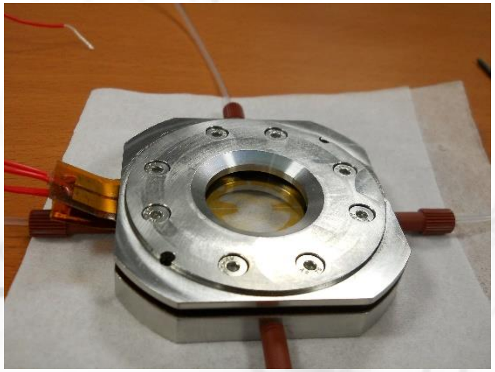
Temperature controlled stage

-180-600°C

And atmospheric control

Diamond Compression Cells

sample flattening



Applications

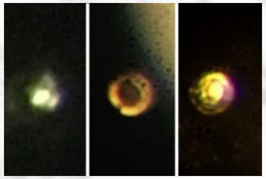
THE QUEST FOR METALLIC HYDROGEN

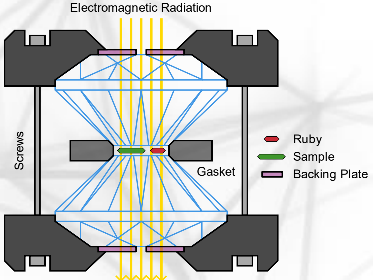
- Wigner and Hungtinton predicted metal hydrogen using quantum mechanics in 1935

- Properties of metallic H_2
  - Superconductor at ambient temperature
  - Possibly metastable
  - Highly energetic rocket propellant: 1700 seconds specific impulse (theory)

- Demonstration
  - Gazeous hydrogen is fully IR transparent
  - Metallic hydrogen is opaque
  - Need to observe progressive closure of the gap in the IR
  - Use of Diamond Anvil Cells,

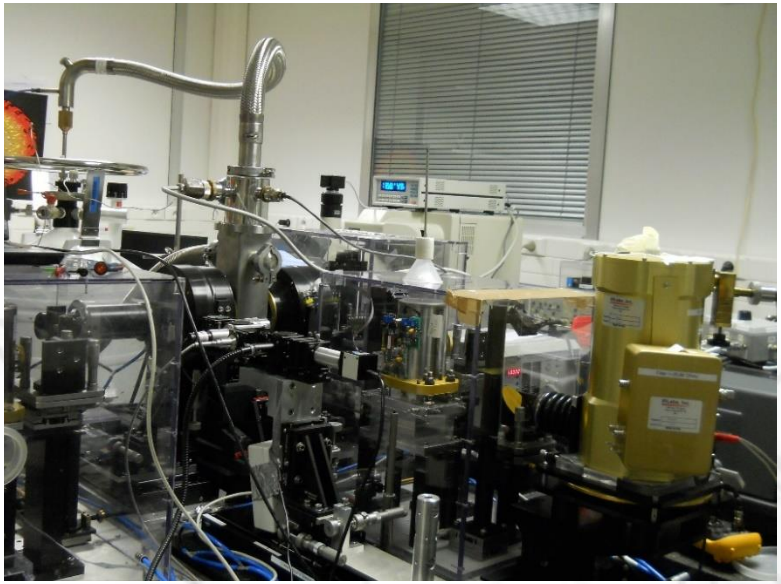
Dias et Silvera
Science 2017

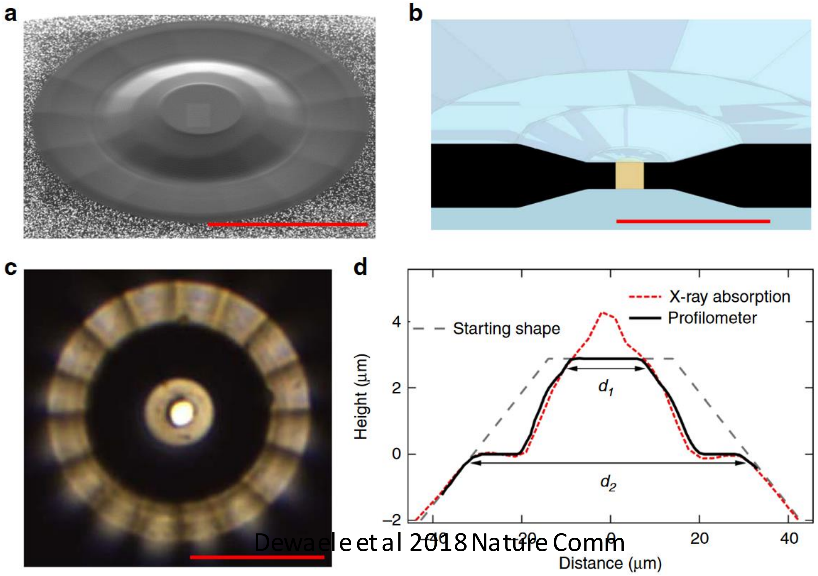




THE QUEST FOR METALLIC HYDROGEN

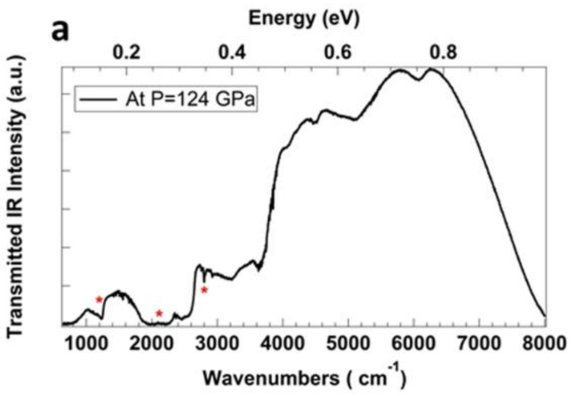
- Instrumental developments:
  - SMIS: development of a specific horizontal microscope
    - 47 mm working distance, 22 µm FWHM IR spot,
    - Transmission down to 2-3 µm holes
  - CEA:
    - Development of new toroidal DAC for up to 600 GPa
    - Metallisation of the joint to avoid H diffusion

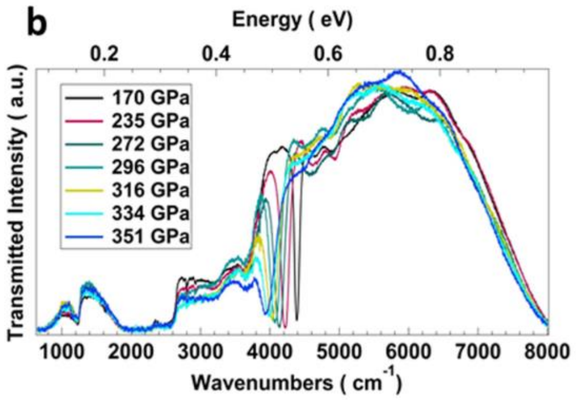


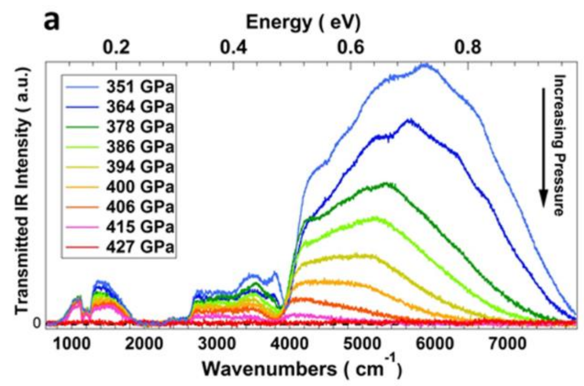


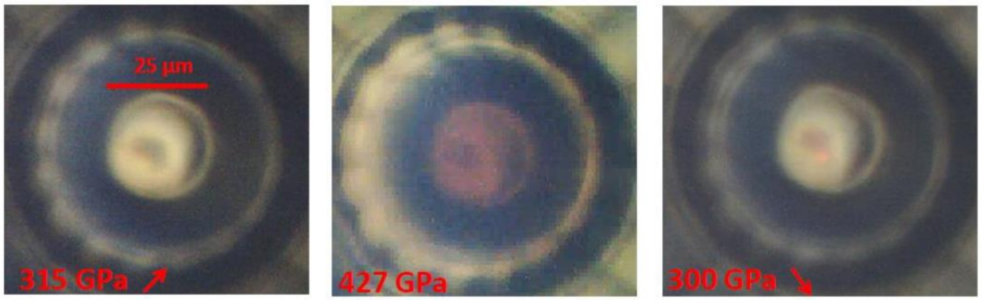
Dewaele et al 2018 Nature Comm

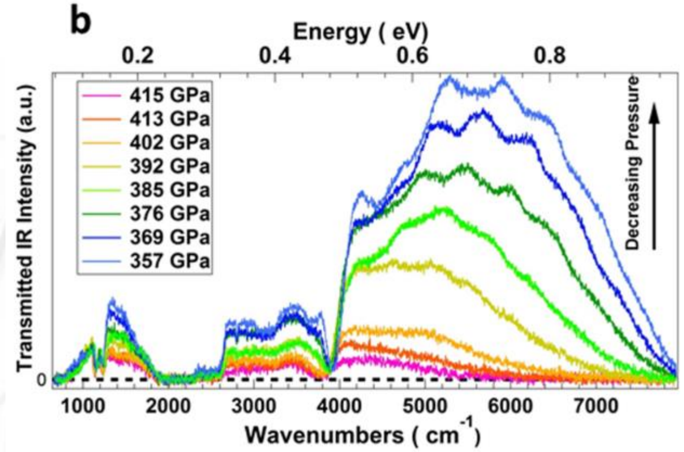
FINALLY









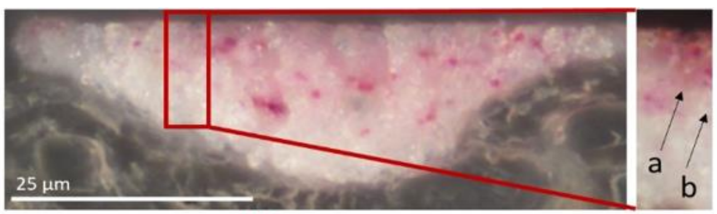


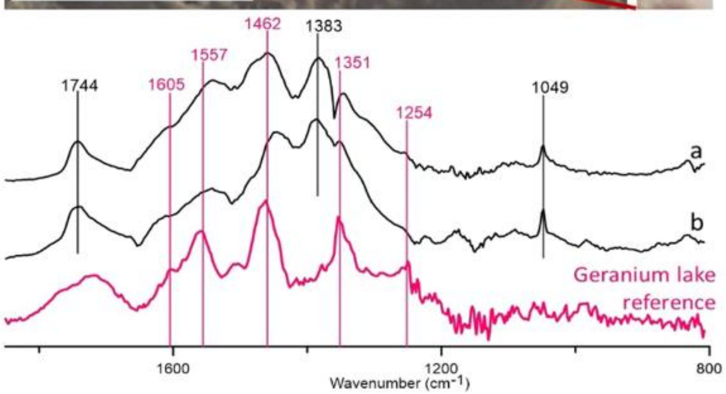
Loubeyre, Occelli, Dumas arXhiv 2018
Loubeyre, Occelli, Dumas Nature 2020, 577,

- Continuous vibron frequency shift
- First order phase transition near **425 GPa/80K** from insulator molecular solid hydrogen to metal hydrogen (4.25Gpa = 4.25 million atmospheres)
- Electronic band gap closure down to 0.5 eV
- Reversible phase transition, back to C2/c-24 phase

Identification of pigment in Van Gogh

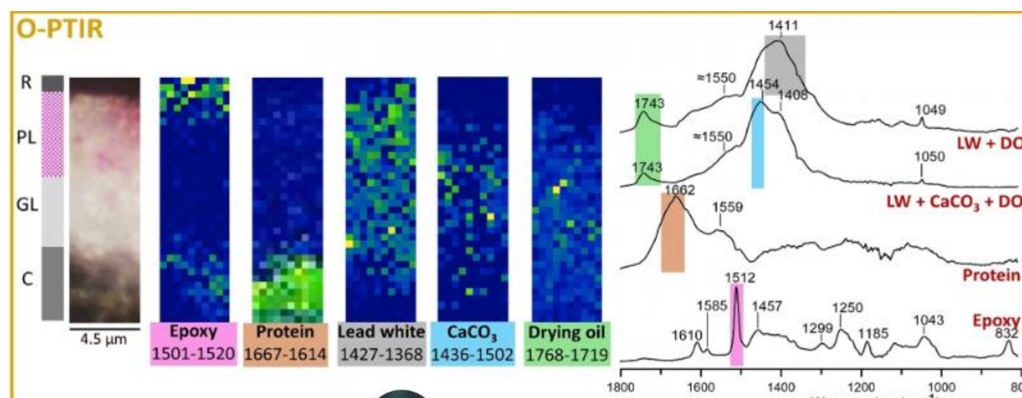
- Multiscale characterization of multilayerd historical paintings
  - Layers < 10µm
- Non destructive
- Identification of pigments, binders, support material,
- Characterization of material aging and degradation pathways

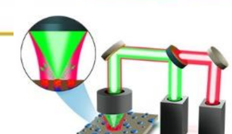




Beltran et al.
2021
Angewandte Chemie

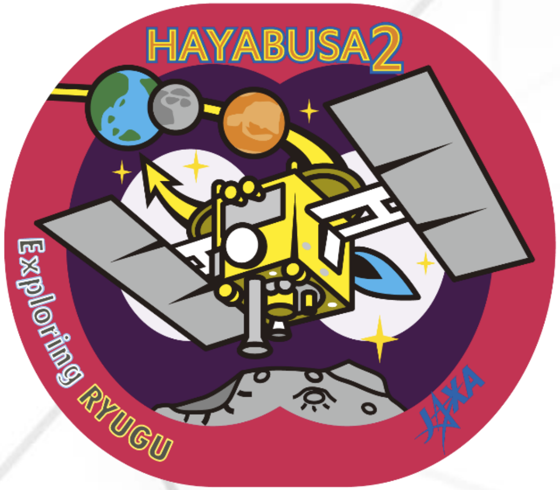
L'Arlésienne
Portrait de madame Ginoux,
Van Gogh, 1888
Kröller-Müller Museum, Netherland

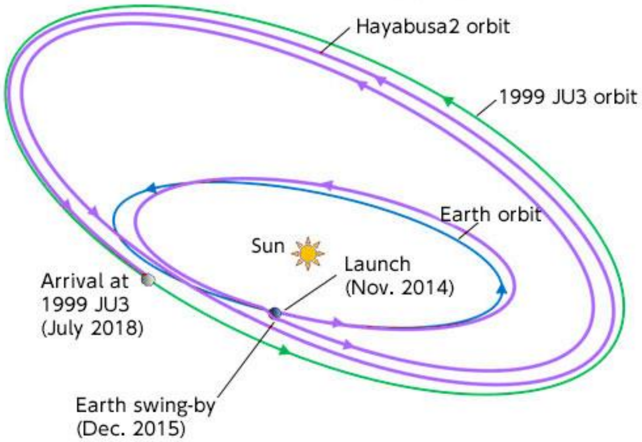




Identification of geranium lake by OPTIR

Ryugu Asteroid sample return mission Hayabusa 2

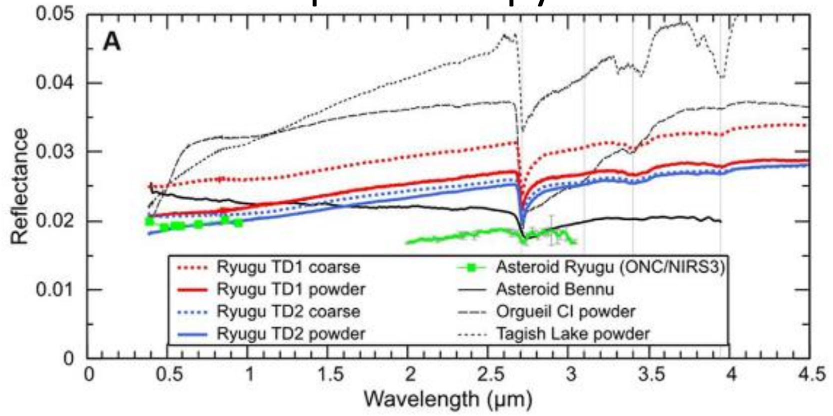




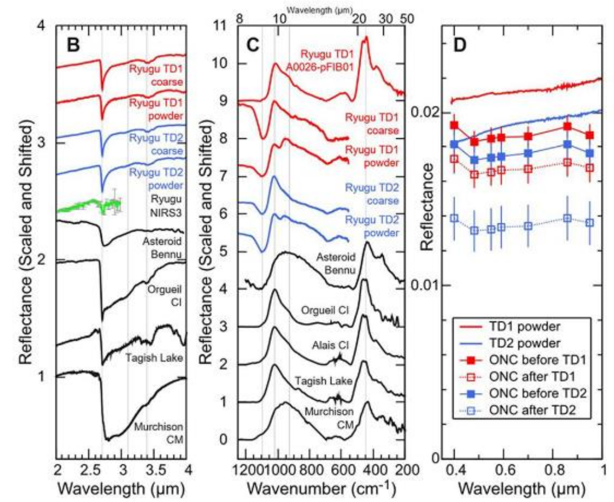
ISMO IJCLab Irène Joliot-Curie Laboratoire de Physique des 2 Infinis IMPMC IAS Orsay

Main findings

Powder spectroscopy



Single grain **micro**spectroscopy



- Ryugu is similar to known CIs with important differences
- Ryugu is very close to Orgueil meteorite
  Existing meteorite collection is biased!
- Ryugu is the new standard for CI

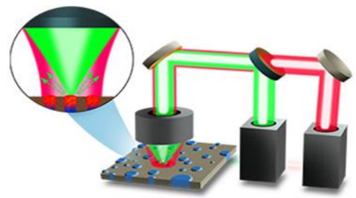
- Ryugu has formed in the far outer system after 2 My and then migrated closer to the sun
- Rich in silicates and carbonates
- 3 My at 50°C: aqueous alterations
- Traces of olivine, pyroxene, amorphous silicates, calcite, and phosphide

T. Nakamura et **[215]**, Science 2022 DOI: 10.1126/science.abn8671.

Beyond the diffraction limit

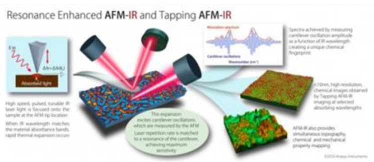
Overcoming the diffraction limit in the mid-IR

O-PTIR
Optical photothermal



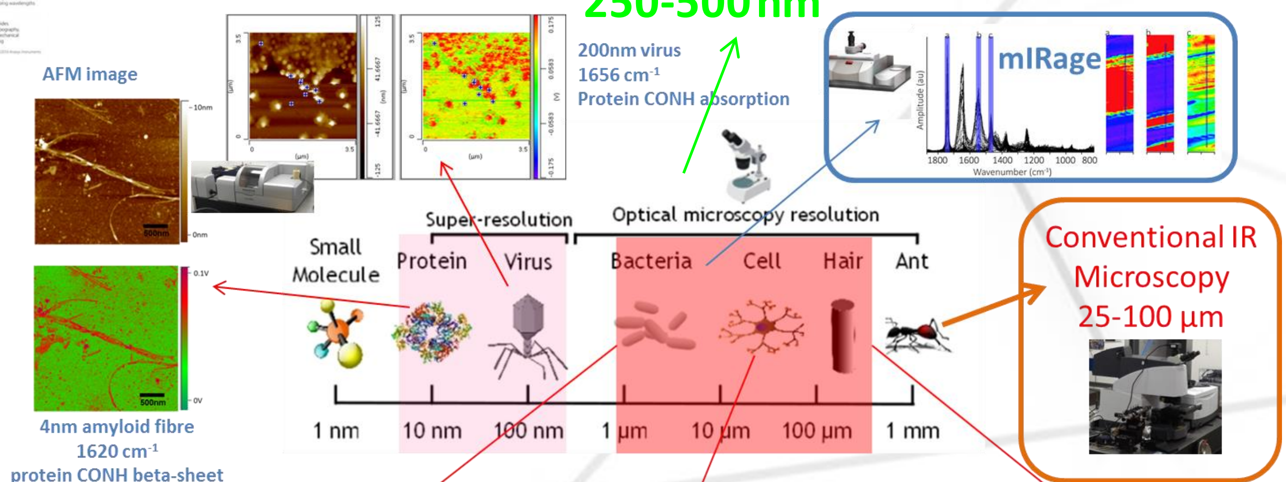
Klementieva et al.
Single nano-amyloid aggregates
Adv Sciences 2021

Nano IR
sSNOM, AFM-IR

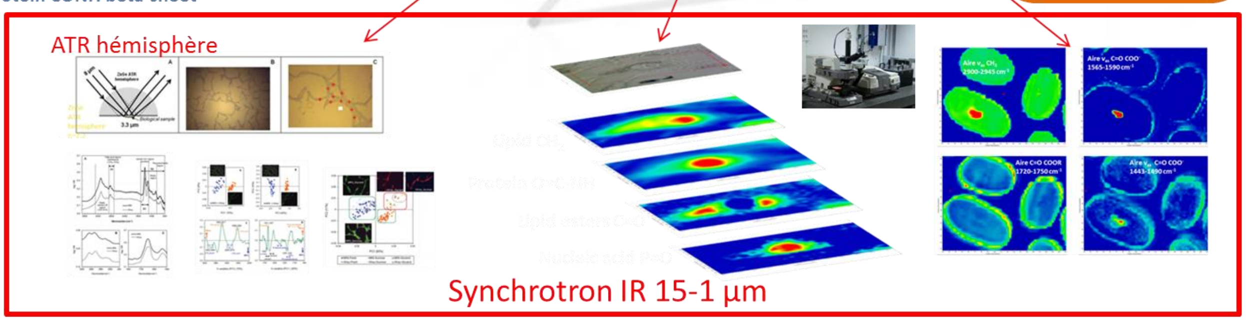


10-50 nm

250-500 nm

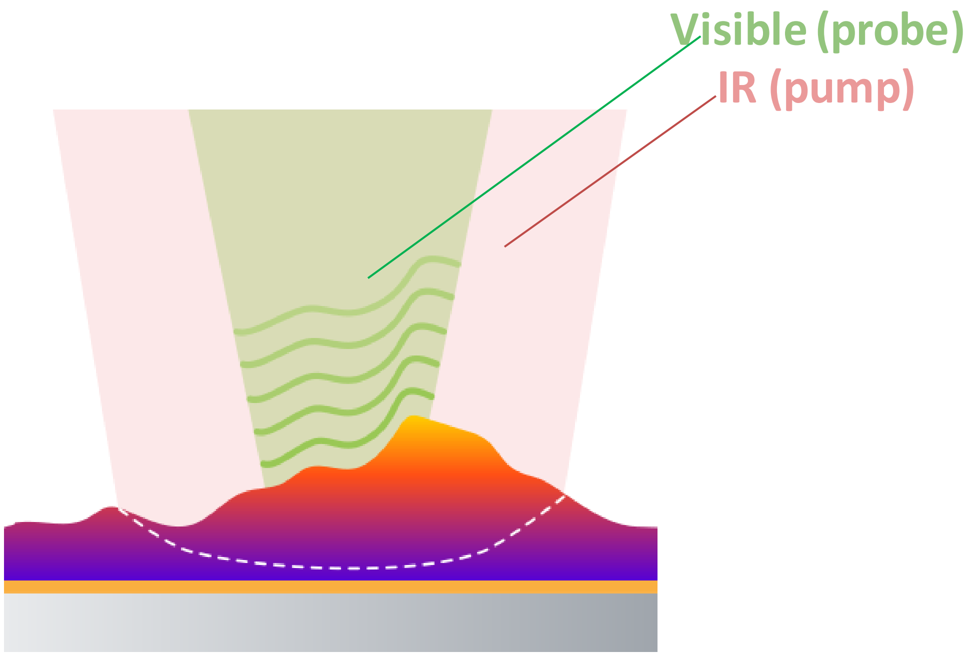


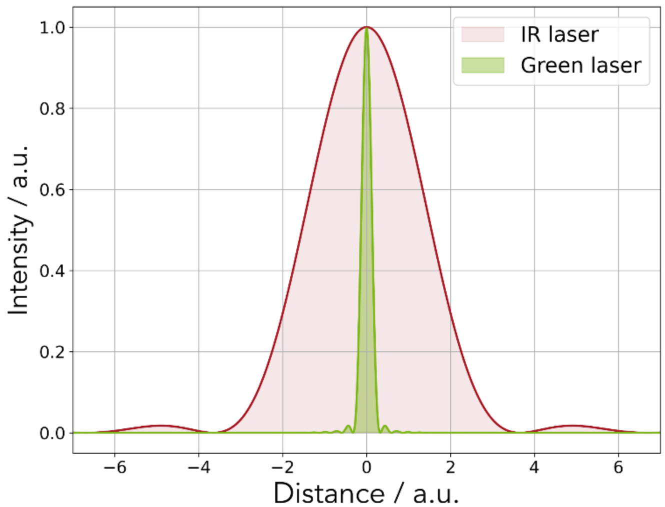
Partouche et al.
Single amyloid fibril
J. Microscopy 2019



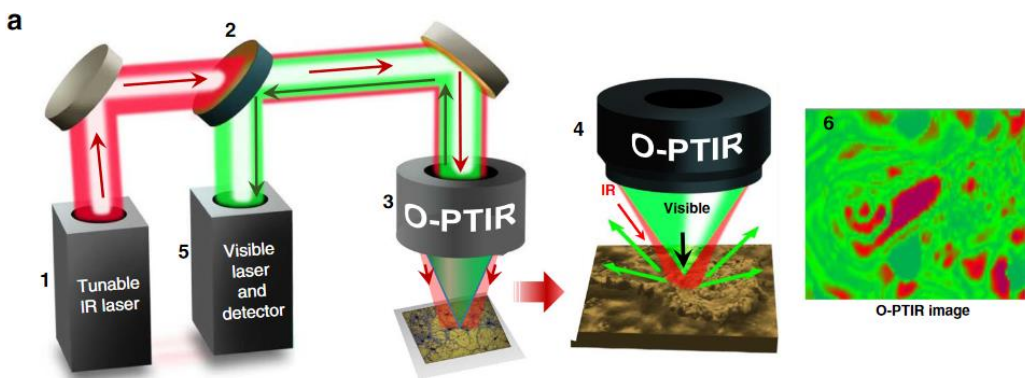
Passot et al.
SR-FTIR of Single bacteria
Analyst 2015

Optical PhotoThermal IR (OPTIR)





Freitas et al 2021



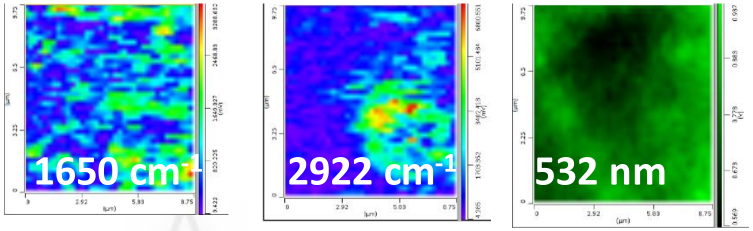
Gustavsson et al 2023

Optical PhotoThermal IR (OPTIR)

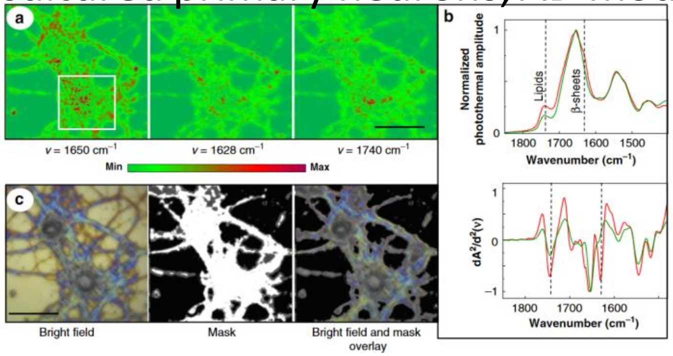
- Optical Photo-Thermal IR (OPTIR)
  - Pump-probe measurement
  - QCL IR pump: excite sample
  - Visible probe: set the spatial resolution
  - Measure changes in sample refractive index and reflectivity
  - 250-500 nm spatial resolution
  - Imaging and spectral modes

- Advantages
  - Spatial resolution
  - Thick samples
  - Compatible with glass slides
  - Measurement in water possible
  - Spectra similar to transmission spectra

Hair sample



β-sheet and lipid oxidation in cultured primary neurons, AD model

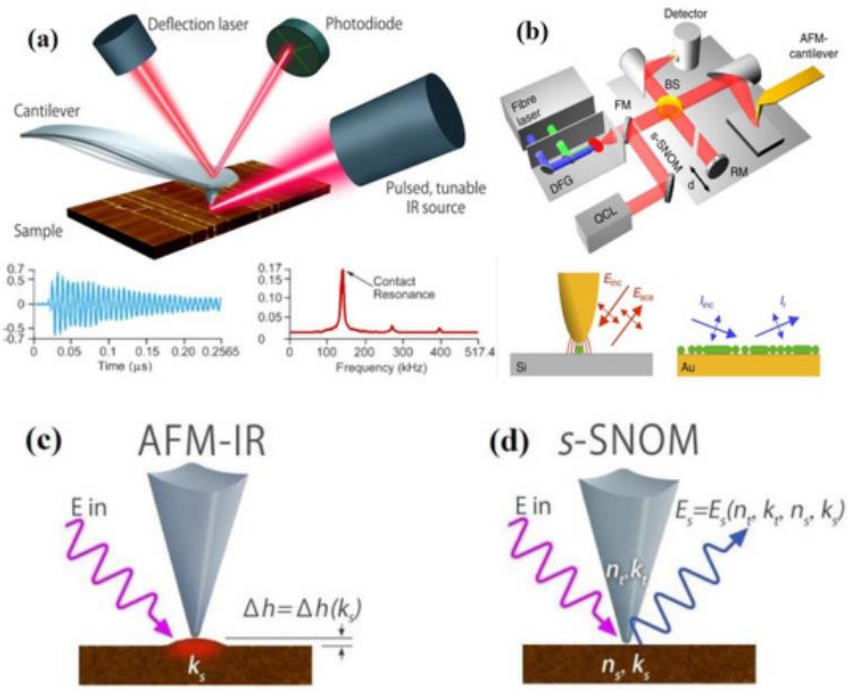


- Disadvantages
  - Restricted spectral range
  - Photodamages
  - Quantification is difficult

Nano-IR spectroscopy

- AFM-IR
  - Photothermal: sample dilatation
  - 10-30 nm spatial resolution
  - Imaging and spectral modes

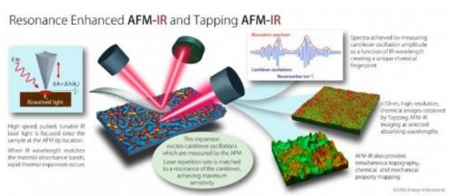
- sSNOM
  - Scattering SNOM: Surface Nearfield Optical microscopy
  - Change in sample reflectivity/refractive index
  - 10-30 nm spatial resolution
  - Imaging and spectral modes

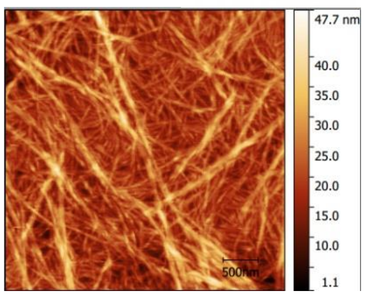


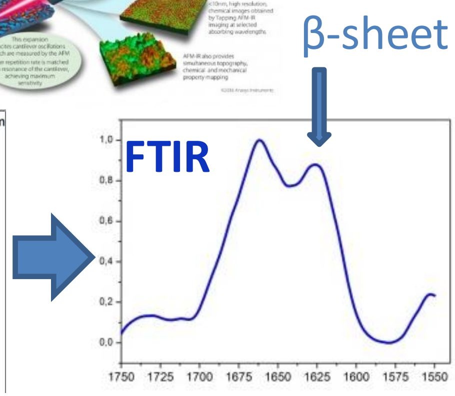
Zhu et al 2021

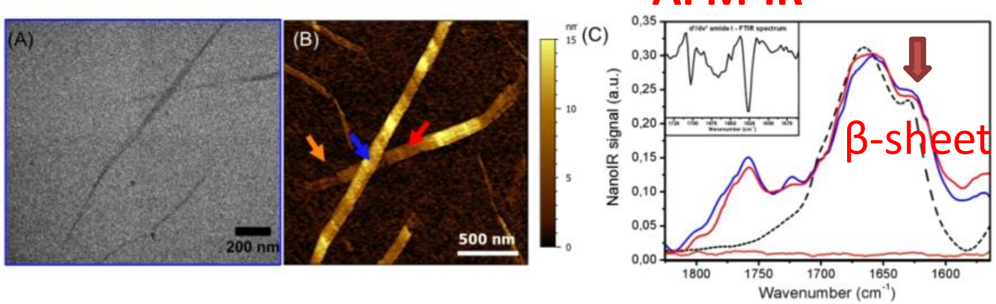
Nano-IR spectroscopy

NanoIR of single amyloid fibrils (bacterial Hfq protein)



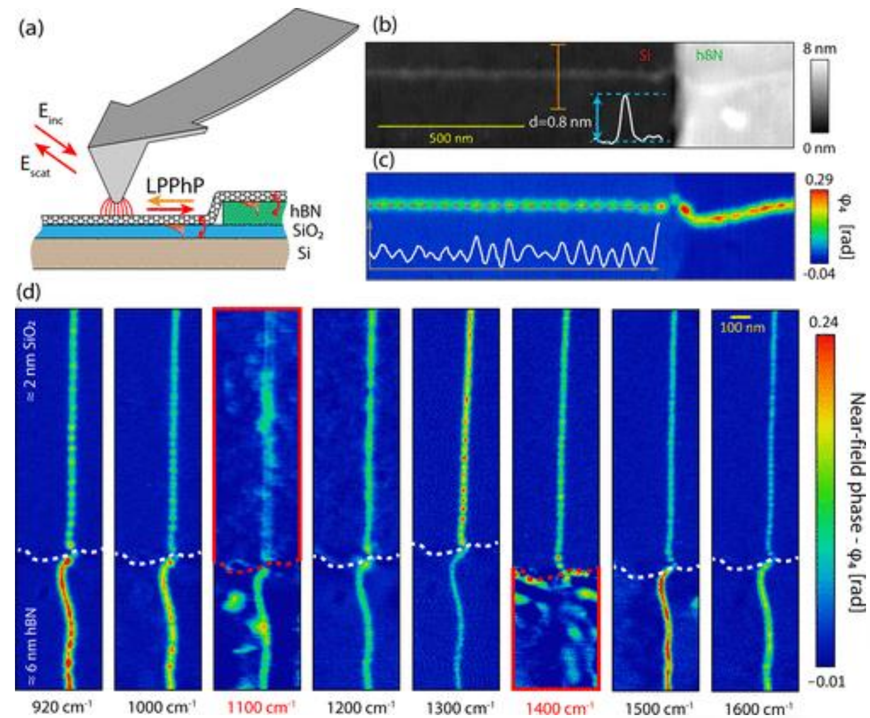






Partouche et al. 2019

Nanotube polariton imaging with sSNOM



Near-field phase images at several different laser frequencies.

Nemeth et al. 2022

Data analysis

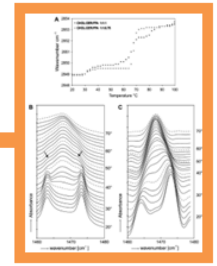
Preprocessing

- Preprocess is used to remove unwanted effects in spectra:
  - Baseline corrections and scatter corrections to remove scattering effects
  - Normalisation to remove thickness effects (scaling)
  - Smoothing to remove noise
- Transformations
  - Kramers Kroenig Transform for specular reflectnace spectra
  - Kubelka Munk Transform for diffuse reflectance data
  - Derivative to enhance the discriminative power
  - Fourier Self Devonvolution to enhance the discriminative power

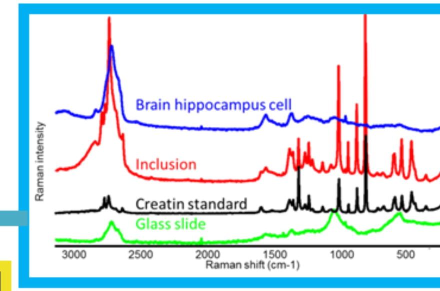
Data analysis (classic)

- PEAK TABLES

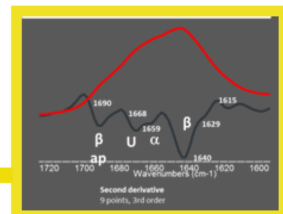
- PEAK PARAMETERS

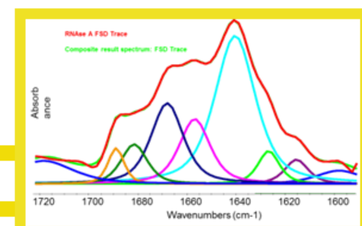


- DIFFERENCE SPECTROSCOPY

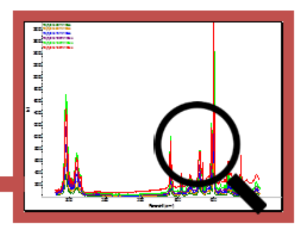


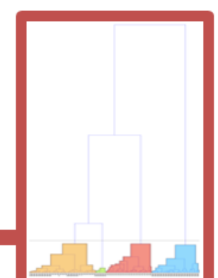
- RESOLUTION ENHANCEMENT
  - DERIVATIVES
  - FOURIER SELF DECONVOLUTION
  - CURVE-FITTING

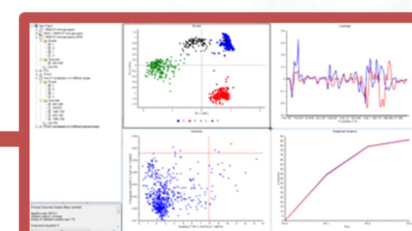




- PATTERN RECOGNITION
  - DATABASE SEARCH
  - CLASSIFICATION
  - DATA STRUCTURE







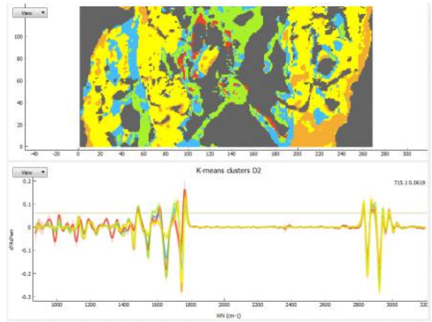
Data analysis : machine learning and multivariate statistics

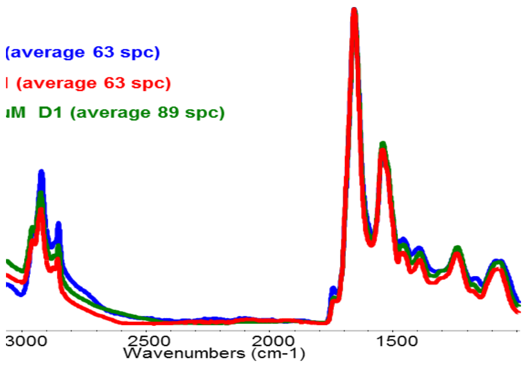
- MULTIVARIATE STATISTICS:
  - Explore and quantify the variability in data (PCA, PLSDA, CVA)
  - Reduce the number of variables (PCA, tSNE, MDS, UMAP…)
  - Remove correlations
  - Quantification in complex systems (PLSR, MCR-ALS…)

- MACHINE LEARNING
  - Unsupervised Clustering (Kmeans Clustering, FCM, HCA,DBSCAN…)
  - Supervised Classification (CART, RFC, XGB…)

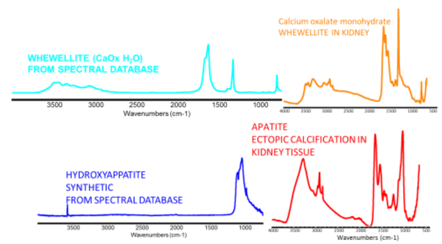
Data analysis challenges

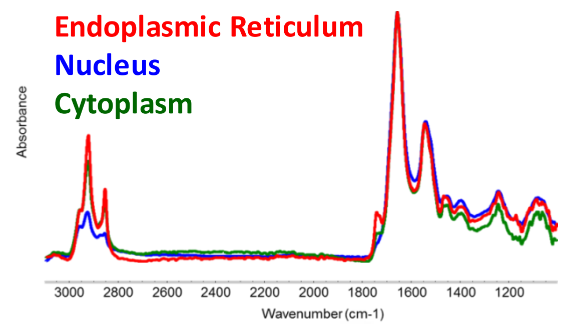
- BIG DATA
  - Tb/day



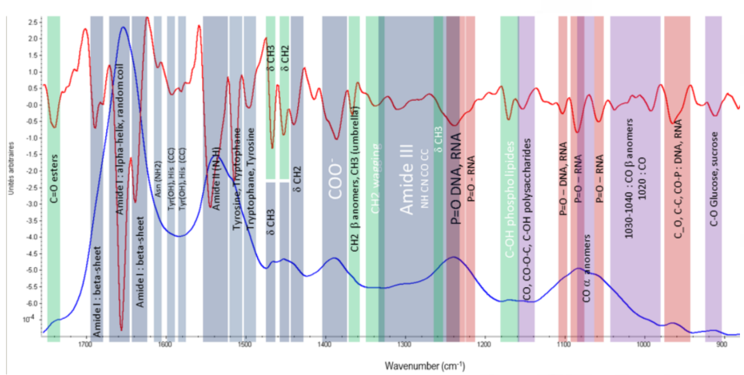


- SMALL RELEVANT VARIABILITY
  - ARTEFACTS & IRRELEVANT VARIABILITY
  - SCATTERING, EFSW …
  - PATIENT VARIABILITY





- SPECTRAL INTERPRETATION
  - COMPLEX SIGNAL
  - EXTRACT RELEVANT MARKERS



The end

Questions?